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13. ABSTRACT ***(Maximum 200 Words)***

This report presents data: 1) On the evaluation of antimalarial drugs, and 2) plasmid DNA and recombinant protein malaria vaccines in the *Aotus/Plasmodium falciparum* and *vivax* model. During the course of these experiments neither Aotus immunized ID with AMA-1, EBA-175 and MSP-1 plasmids alone or in combination with or without aGMCSF were protected against a *P. falciparum* FVO challenge A C2A clone *P. falciparum* was adapted to intact and splenectomized *Aotus*. Chloroquine resistance reversal was achieved in *Aotus* infected with the AMRU-1 strain of *P. vivax* by using chloroquine and prochlorperazine in combination. Oligodeoxynucleotides when given intramuscularly to *Aotus* improved immunogenicity of a *P. falciparum* PADRE 45 peptide immunogen. A significant degree cross-protectior was developed in FVO immune *Aotus* that were challenged with an heterologous CAMP strain of *P. falciparum*. Immunization with a plasmid encoding region II of EBA-175 followed with a recombinant protein boost, partially protected Aotus monkeys against *P. falciparum* challenge. Both Artelinic Acid and Artesunic Acid at 8-32 mg/kg orally for five days were effective at clearing parasitemia in *P. falciparum* FVO inoculated Aotus. *Aotus* immunized with plasmids and/or recombinant $MSP1_{42}$ were partially protected against *a P. falciparum* FVO challenge.

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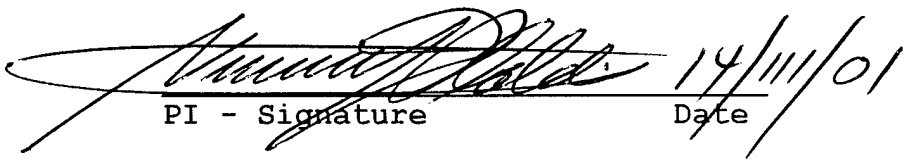

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INTRODUCTION:

Each year there are 300-500 million new infections and 2-5 million deaths attributable to malaria that occur primarily in countries in the tropics, particularly in sub-Saharan Africa **(4)**. During the past 10-20 years the malaria problem has intensified in some parts of the world because parasites have developed resistance to drugs used for treatment and prevention; the anopheles mosquito, which transmits the parasite to humans, has developed resistance to insecticides, and control efforts have been reduced as resources have diminished in some developing countries **(7)**.

The use of *Aotus lemurinus lemurinus* (Panamanian *Aotus* monkey), kariotypes VIII and IX **(16)** as a model to study malaria drug resistance and vaccine efficacy, have been ongoing at Gorgas Memorial Laboratory since 1976, due in part to the availability of this monkey in Panama **(20)**, and also to the increasing drug resistance exhibited by the highly pathogenic *Plasmodium falciparum* parasites in Asia, Africa, and Latin America, and more recently *Plasmodium vivax* in the Melanesian and Indonesian archipielago **(21)**. Previously, Schmidt **(26,27)** used the Colombian *Aotus* as the experimental host for antimalarial drug studies, but embargoes imposed by South American countries on the exportation of monkeys in the mid 1970's seriously restricted the use of *Aotus* for biomedical research in the United States, and in 1976 the project was transferred to Gorgas Memorial Laboratory where Panamanian *Aotus* were available for research. Five strains of *P. falciparum*, Vietnam Smith, Uganda Palo Alto , Vietnam Oak Knoll (FVO) , Santa Lucia **(5)**, and a C2A mefloquine resistant clone, and three strains of *P. vivax* Chesson (chloroquine sensitive), New Guinea AMRU-1 (chloroquine resistant) and Sal-1, have been adapted to Panamanian Aotus.

These strains exhibit diverse susceptibility and/or resistance to standard antimalarial agents. The course of untreated infections in Panamanian *Aotus* has been characterized and compared with that in *Aotus* of Colombia **(25)**. Overall, the virulence of these strains was less in Panamanian than in Colombian owl monkeys, as indicated by lower mortality rates of Panamanian monkeys during the first 30 days of patency. Maximum parasitemias of the Vietnam Smith and Uganda Palo Alto strains were, however, significantly higher during the first 15 days of patency in Panamanian than in Colombian owl monkeys. These quantitative differences in infection parameters between Panamanian and Colombian owl monkeys have not invalidated the use of the former for evaluation of new antimalarial drugs.

Numerous candidate antimalarial drugs of diverse chemical classes have been evaluated against trophozoite-induced infections of one or more *P. falciparum* strains during the course of these contracts. In seeking alternatives to primaquine, two 8-aminoquinolines proved to be active against the blood stages of *P. falciparum* **(2, 18)**. Desferrioxamine, an iron-specific- chelating agent, was shown to suppress parasitemias of the virulent

Uganda Palo Alto strain of *P. falciparum* **(23)**. The *in vitro* activity of two halogenated histidine analogs was not confirmed by evaluation against *P. falciparum* infections in owl monkeys **(22)**.

Chloroquine-resistance of *P. falciparum* represents the greatest challenge in developing effective antimalarial drugs. Reversal of chloroquine-resistance in *P. falciparum, in vitro*, was achieved by the co-administration of verapamil (a calcium channel blocker) plus chloroquine **(17)**. Other in vitro studies have shown that there is a significantly greater efflux of chloroquine from erythrocytes containing falciparum parasites resistant to chloroquine than from red cells parasitized by chloroquine-sensitive falciparum malaria **(14)**. Calcium channel blockers appear to prevent this active efflux of chloroquine, thus allowing the drug to accumulate to parasiticidal levels.

Based upon the success of *in vitro* reversal of chloroquine-resistance, trials were initiated to determine if resistance could be reversed in *Aotus* infected with the chloroquine-resistant Vietnam Smith strain of *P. falciparum*. Six calcium channel blockers, or similarly acting drugs, were co-administered with chloroquine in diverse regimens. The desideratum of chloroquine-resistance reversal was administration of a single course of treatment, with parasite clearance and infection cure. Suppression of parasitemia was obtained during an initial course of treatment, but parasite clearance and cure occurred in some instances only after re-treatment. Such infection parameters were similar to those in monkeys with self-limited infections and cure could be attributed to acquired immunity.

Limited trials with desipramine, Norpramin , a tricyclic psychotropic drug, demonstrated the feasibility of reversing chloroquine-resistance in vivo **(1)**. parasite clearance was obtained, but the infection was not cured.

Subsequently, in vivo reversal of chloroquine resistance was obtained with combinations of chloroquine plus chlorpromazine or prochlorperazine. Such reversal was exhibited by rapid suppression and clearance of parasitemia, resulting in infection cure without retreatment **(15)**.

Evaluation of two oil-soluble derivatives of artemisinin, artemether and arteether, demonstrates that both possess similar activity to cure infections of a multi-drug resistant *P. falciparum* strain in *Aotus* **(28)**.

Some strains of *P. vivax* from Melanesia and the Indonesian archipelago have demonstrated resistance to treatment with chloroquine **(19, 24)**. Unlike chloroquine-resistant falciparum malaria, there exists no easy alternative to chloroquine-resistant strains of vivax malaria. Using WR 238605 alone or in combination with chloroquine in Panamanian *Aotus* monkeys it was demonstrated that WR238605 is a an alternative treatment for chloroquine-resistant vivax malaria **(21)**. The compound WR 238605 is a primaquine analog developed by the US Army as a better tolerated, more effective replacement for primaquine. Recent studies done at Gorgas Institute with Artemisin derivative durgs developed by the U.S. Army such as Artelinic acid demonstrated its efficacy against the FVO strain of *P. falciparum* when administered orally to *Aotus l. lemurinus.*

Both the purpose and methods of approach of the present work remains essentially unchanged since 1976, viz to ascertain the antimalarial activity of drugs against *P. falciparum* and *P. vivax* in *Aotus*. The method of approach may vary on an ad hoc basis, such as administering a combination of drugs.

The long term goal of the second part of this project is to develop fully protective plasmid DNA vaccines that induce protective immune responses against the sporozoite, liver and erythrocytic stages of *P. falciparum*. If successful, it will establish, for the first time, that plasmid DNA vaccines can protect non-human primates, a critical step forward for the use of plasmid DNA vaccines in humans.

Vaccines are aimed at inducing immune responses that disrupt the complex cycle of the parasite at one more points: anti-sporozoite antibodies that prevent invasion of hepatocytes; cytotoxic T lymphocytes, cytokines, and antibodies that eliminate infected hepatocytes; antimerozoite antibodies that prevent invasion of erythrocytes; antibodies that neutralize parasite exoantigens known to induce harmful cytokine responses; antibodies that attack infected erythrocytes; cytokines that kill parasites within erythrocytes; and, anti-sexual stage antibodies that prevent the development of sporozoites in the mosquito.

Previous trials of malaria blood stage vaccines have shown that the Panamanian *Aotus\P. falciparum* model to be suitable for this purpose. **(8-10)**.

Immunogenicity studies of a plasmid DNA vaccines encoding the circumsporozite *P. yoelli* rodent malaria gene (PyCSP) in Panamanian *Aotus* monkeys demonstrated that the intradermal route of inoculation (ID) induces a higher level of antibodies than the intramuscular route (IM). Antibody levels induced in this manner reached a peak at week 9 and titers declined to 50% their peak value by week 14. When boosted at week 46 antibody levels increase 4 fold by week 49. This was comparable to antibodies generated with a Multiple Antigen synthetic peptide vaccine (MAP) delivered with an adjuvant **(4)**

We have used this inmunization scheduled to test single or multi-gene DNA plasmid vaccines in *Aotus* monkeys. Additionally we have tested the ability of recombinant cytokines to enhance the immunogenicity and protective efficacy of the DNA vaccines. Preliminary using a small group of *Aotus l. lemurinus* (n = 3) demonstrated partial, but incomplete, protection with a DNA vaccines for either AMA-1 or EBA-175 alone. These studies indicated that animals which received the vaccine candidates, had a short, but apparent significant delay in the onset of parasitemia {approximately 33% (1 of 3) self-cured, whereas none of the control animals did}. However, since the number of animals per group in each of these pilot studies were small, it was not possible to determined the absolute efficacy of these candidate vaccines, but these experiments suggested to the investigators that further studies were warranted. MSP-1, when used as a

but apparent significant delay in the onset of parasitemia {approximately 33% (1 of 3) self-cured, whereas none of the control animals did}. However, since the number of animals per group in each of these pilot studies were small, it was not possible to determined the absolute efficacy of these candidate vaccines, but these experiments suggested to the investigators that further studies were warranted. MSP-1, when used as a protein/peptide vaccine formulation, provided protection from a *P. faciparum* infection in *Aotus* monkeys and we have demonstrated that, in mice and in Rhesus monkeys, the cytokine GM-CSF augmented both immunogenicity of a malaria DNA vaccine (personal communication. W. Weiss). We have now completed a pilot experiment to determine if *Aotus* Granulocyte-Macrofage-Colony Stimulating Factor (aGM-CSF) can augment immunogenicity and protective efficacy of a multi-gene erythrocytic vaccine.

In addition, synthetic oligodeoxynucleotides containing CpG motifs enhance immunogenicity of a peptide malaria vaccine when tested in Panamanian Aotus **(11)**. Different vaccine formulations, routes and methods of administration with a comparable Hepatitis B Plasmid DNA vaccine were explored in Panamanian Aotus in order to elucidate the best route and methods of immunization for a plasmid DNA malaria vaccine **(6)**. Further studies with single or multistage antigen plasmid DNA vaccines have been conducted or are in progress in Panamanian Aotus with variable results. Herein, we report partial protection obtained in Aotus monkeys immunized with either plasmid or recombinant protein in a primary and boosting immunization schedule using $MSP1_{42}$ as an antigen.

We have also tested the effect of prior *P. falciparum* infection on the immunogenicity of a DNA vaccine, obtaining partial protection in 67% of the monkeys **(12)**. Also, evaluated in Aotus monkeys the characteristics of *P. falciparum*-induced anemia in two different experimental settings and hypothesis that a non-antibody/non-complement-mediated lysis of uninfected erythrocytes was the principal cause of anemia, and that bone marrow suppression and lysis of infected erythrocytes contributed to the anemia **(13)**. In addition, we tested the hypothesis that *a P. falciparum* ligand, EBA-175 region II (RII), can be used as an immunogen in Aotus to induce antibodies that block the binding of RII to erythrocytes and thus inhibit parasite invasion of erythrocytes **(29)**.

The purpose of this report is to: 1) Present data on the evaluation of potential antimalarial activity of drugs in the pre-clinical model of *Aotus l. lemurinus* (Panamanian night monkey) experimentally infected with *P. falciparum* or *P. vivax*, and 2) data on plasmid DNA and recombinant protein malaria vaccine experiments. These studies were supported by the U.S. Army and the U.S. Navy Malaria Programs.

BODY:

I. Experimental Methods

The first aim of this project is to evaluate the potential antimalarial activity of drugs, or combination thereof, in the preclinical model of *Aotus* experimentally infected with *P. falciparum* (or *P. vivax*). Specifically, the vertebrate host is *A. l. lemurinus*, the Panamanian night monkey. These animals are either feral, laboratory adapted or laboratory born. No naturally acquired, human plasmodium infection has been reported in *Aotus*. The Vietnam Smith/RE strain of *P. falciparum* was adapted to *Aotus* of Colombian origin in 1971 **(26)** and in Panamanian *Aotus* in 1976. **(25)**. The course of untreated infections, essential for comparison with treated infections, has been documented in Panamanian *Aotus* **(25)**. This plasmodium strain is resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine **(27)**.

To initiate an experiment, infected blood (with 2.5% sodium citrate as the anticoagulant) from an untreated *Aotus* was diluted appropriately in chilled saline (0.85%) or RPMI, such that each milliliter contained 5,000,000 parasites. This amount was inoculated into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm. **(3)**

Blood films from untreated *Aotus*, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If recrudescence occurred, blood films were obtained again on a daily basis.

Oral administration of drugs was by gastric intubation with a 14 French catheter. The total volume of fluid administered, drug solution or suspension, and rinse was 14 ml.

Response to treatment was categorized as clearance and cure, clearance and recrudescence, or suppression withouth clearance. The day of clearance was defined as the first of three consecutive days in which the thick blood films were parasite negative. The day of recrudescence was the first of three consecutive days of positive thick blood films after a period of clearance. Suppression was defined as a transient decrease in the parasite count post-treatment without clearance.

The second objective of this project is to evaluate plasmid DNA vaccines against the blood and sporozoite stages of *P. falciparum* and against the blood stages of *P. vivax* in the Panamanian *Aotus* model. To this end we have evaluated single and multigene DNA vaccines of *both P. falciparum* and *P. vivax* with or without the addition of cytokines. The results of these experiments are detailed in results.

II Results

1. Passage of *P. falciparum* Smith/RE strain

In order to bring up a frozen strain of Smith/RE *P. falciparum,* two malaria naive monkeys were inoculated intraperitoneally (IP) with blood from two different donor monkeys on 28 August 1996. Both animals remained negative for more than sixty-four days.

2. Reversal of Chloroquine resistance of *P. vivax* AMRU-1 strain.

Previous studies with a CQR *P. falciparum* have shown that it is possible to achieve *in vivo* reversal of CQR by the co-administration of prochlorperazine and chloroquine, as evidenced by infection cure. Neither drug alone affects such cure.

This study was designed to determine if CQR of the AMRU-1 strain (P. vivax) can be reversed in vivo by prochlorperazine plus chloroquine.

On 21 October 1996, each of 10 *Aotus l. lemurinus*, cured of *P. falciparum*, was inoculated intravenously with 5 x 10^6 AMRU-1 strain parasites of *P. vivax*, and divided into three groups of three monkeys plus a single untreated control, to determine if the co-administration of prochlorperazine (WR 280003 AC; BN 43106) and chloroquine (WR 1544 BM; AR 20613) against infections of the AMRU-1 strain (CQR) of *P. vivax* will reverse chloroquine resistance. As shown in Table 1-2, Prochlorperazine alone at 20 mg/kg x 7 days did not have any effect on 3/3 animals from Group 1. Animals from this group cleared 18 and 37 days post inoculation (PI). One animal of this group died of malaria 20 days PI and the animal which cleared 18 days PI had a transient two days recrudescence 4 days

after clearance. The two surviving animals remained negative for more than 61 and 74 days respectively. Group 2, that received Prochlorperazine 20 mg/kg plus chloroquine 10.0 mg/kg cleared their parasitemias 4-7 days PI without recrudescence for more than 87-89 days. In group 3, that received Chloroquine 10.0 mg/kg 2/3 monkeys cleared parasitemias 3-8 days PI without recrudescence remaining negative for more than 84-88 days PI. Although animals from this group, one died of malaria 8 days after inoculation.

A striking finding during the course of this experiment was the anemia related deaths observed in two monkeys and that three had to be transfused with fresh whole blood due to their extremely low hematocrits. It is postulated from these findings that another cause different than *P. vivax* AMRU-1 infection might have been the cause of death in these animals.

In vivo reversal of CQR of the AMRU-1 strain by the co-administration of prochlorperazine could not definitively demonstrated with a 7 day course treatment in this experiment.

3. Adaptation of *in vitro* cultured Mefloquine and Atovaquone:Malarone resistant strains of *P. falciparum* to Aotus monkeys.

In an attempt to adapt *in vitro* cultures of a Mefloquine (Mef 2.5) and an Atovaquone (C2B) resistant strains of *P. falciparum*, two malaria naive splenectomized monkeys were inoculated intravenously (IV) on 27 January 1997, with 2 mls of packed red cells from room temperature *in vitro* culture parasites. No parasites were detected in daily blood smears for more than 42 days PI.

4. Passage of *P. vivax* AMRU-1 strain.

On 15 October 1996, one monkey was inoculated intraperitoneally (IP) for passage of a frozen strain of AMRU-1 *P. vivax* malaria. The monkey never developed a detectable parasitemia and remained negative for more than 75 days PI.

5. Passage of *P. vivax* Sal-1 strain.

To bring up a frozen strain of Sal-1 *P. vivax*, two *P. falciparum* cured monkeys, one intact and one splenectomized, were inoculated IP on 2 and 18 October 1996. Both animals remained negative for more than 118 and 121 days respectively.

6. Efficacy of a *P. falciparum* AMA-1 Erythrocytic DNA vaccine in Aotus monkeys.

Nine malaria naive *Aotus* monkeys divided into 3 groups of 3 monkeys, were vaccinated intradermally with four doses of a plasmid DNA encoding AMA-1 with or without lipid MPL They were challenged with 1 x 10^5 parasites of the *P. falciparum* FVO strain on 19 September, 1996. All vaccinated and control animals were patent by day 7 PI with a prepatent period ranging from 3-6 days as shown in table 3. Control animals were treated on day 12 PI and treatment was initiated in all vaccinated animals between days 13-15 PI. Except for one animal of Group 1 (Monkey 12770) which maintained parasitemia levels under 150,000 parasites/ul, all of the remaining animals had steadily increasing parasitemias that reached the 300,000 parasites/ul treatment threshold. However, its hematocrit had a 40% reduction during the course of parasitemia and had to be treated with mefloquine . During the course of this experiment two monkeys died. One due to aspiration pneumonia during oral mefloquine treatment and another (12788) to malaria, 39 days PI.

On January 7, 1997 all of the remaining monkeys were re-challenged with 10,000 parasites of a *P. falciparum* FVO strain. This time, as shown in Table 4, infection in all monkeys were patent between days 7-8 PI. Parasitemias were below 100,000 parasites/ul, but their hematocrits suffered a significant reduction by day 22 PI, when two animals 12770 and 12792 had to be treated with mefloquine. By day 24 PI, three other monkeys 12790, 12791 and 12793 had to be treated as well. Albeit, monkey 12787 from Group 1 and 12789 from Group 2 had a parasitemia course below 10 parasites/ul, the former had to be treated 29 days PI and the latter self cured.

7. Efficacy of *P. falciparum* EBA-175 DNA vaccine in *Aotus* monkeys.

To test the efficacy of *P. falciparum* EBA-175 erythrocytic plasmid DNA vaccine, nine naive *Aotus* were divided into three groups of 3 monkeys and vaccinated intradermally with 500 ug of plasmid encoding the EBA-175 and P2P30 tetanus toxin protein repeat. On 7 January 1997, all animals received 1 x 10^5 parasites of the FVO strain of *P. falciparum* . As seen in Table 5, by day 6 PI all had patent infections. Treatment with mefloquine was initiated between 11-15 days PI in all animals, except for monkey 12811 in Group 2 and control animal 12813 which by that time had not reached the 300,000 parasites/ml mark. However, by day 20 the hematocrit of monkey 12813 was 20% and had to be transfused with whole blood. This animal died the next day. Monkey 12811 which Hto remained over 30% during the course of infection, self cured 21 days PI.

8. Immunogenicity of a PfCSP MAP Vaccine in *Aotus*

Linear and Multiple Antigen Peptides (MAP) sequences derived from the PfCSP protein of *P. falciparum* , were synthesized as peptide sequences with an exogenous T-cell helper epitope (P2P30 or PADRE). These synthetic peptide sequences were incorporated into a liposome vaccine formulation and delivered IM with Alum. The purpose of this experiment was to test the relative immunogenicity of these vaccine candidates in a primate model.

On January 9, 1997, thirty *P. falciparum* and *vivax* double cured Aotus monkeys were divided into six groups of 6 monkeys each and vaccinated with synthetic peptides derived from the PfCSP sequence in different peptide/helper formulations with monophosphoryl lipid A. Each monkey was inoculated IM in the the quadriceps muscle, with 400 ul total volume; (200 ul/site). All animals received 100 ug of antigen per dose and will be immunized three times at monthly intervals. Serum collection for antibody determinations was carried out every two weeks until 26 June 1997. No parasite challenge was carried out in this experiment.

9. Immunogenicity studies of a MAP vs Linear NANP vs NANPNVDP Malaria peptide vaccine in *Aotus*.

On 5 August 1996 a total of 18 malaria double cured *Aotus l. lemurinus* monkeys were divided into 6 groups of 3 monkeys each and immunized IM in the bilateral quadriceps (200 ul each) with a dose of 100 ug in 400 ul of a Peptide vaccine formulation as follows:

Group 1 monkeys were immunized with a Linear (NANP)6 P2P30 peptide. Group 2 with a Linear (NANPNVDP)3 P2P30 peptide. Group 3. with an MAP4 (NANP)6 P2P30 peptide. Group 4 with an MAP4 (NANPNVDP)3 P2P30 peptide. Group 5 with a PADRE-PFB (aKXVAAWTLKAa(NANP)4-GGS) peptide and Group 6 was inoculated with alum as a Control. All animal were inmunized three times and bled five times at monthly intervals. No challenge was carried out in this experiment and it was completed on 20 December 1996.

10. DNA-based immunization of *Aotus* against HBsAg

In order to elucidate why the IM route using a PyCSP malaria DNA vaccine was not effective in *Aotus* as has been previously reported (4), a HsBAg hepatitis DNA vaccine known to be immunogenic by the IM route in *Macaca mullata* monkeys, was chosen as an antigenically distinct vaccine. Forty *P. falciparum* and *vivax* double cured *Aotus* known to be negative to HsBAg hepatitis antibodies, were divided into 10 groups of 4 monkeys each, and vaccinated using either the IM, ID or Intranasal routes. Vaccine formulations consisted of saline, liposome and oligonucleotides or a combination of one or all of them. The positive control group was

vaccinated with a commercial recombinant HsBAg protein vaccine. All monkeys were bled 7 times for HsBAg antibody level determination and three times for lymphocyte collection which were used in cellular immunity studies. In addition, on 27 September, 1996 all animals received a recombinant HsBAg protein booster. Immunogenicity studies are in progress. This experiment ended on 20 December 1996. The addittion of oligonucleotides to the vaccine formulation greatly increased the antibody responses observed with this antigen.

11. DNA Immunization with CSP, SSP2 and Exp-1 *P. falciparum* pre-erytrocytic vaccine and challenge.

On July 17, 1996, 28 malaria naive lab-born monkeys, previously vaccinated with 4 doses of a CSP, SSP2, and EXP-1 plasmid DNA pre-erythrocytic vaccine, were challenged with 21,300 sporozoites of the Santa Lucia strain of *P. falciparum.* All monkeys were splenectomized 14,15 and 16 days later and tissue samples, tissue impression smears and samples for PCR were collected. Daily thick blood films, taken for more than sixty days, were negative. In addition bi-weekly blood sampling for PCR malaria detection were also negative. Spleen impresion smears taken during splenectomies did not reveal any parasites.

12. Adaptation of a *P. falciparum* strain 1088 to Panamanian *A. l. lemurinus* monkeys.

In an attempt to adapt a *P. falciparum* 1088 strain to Panamanian *A. l. lemurinus* one malaria naive splenectomized monkey was inoculated intraperitoneally (IP) with frozen blood sent from WRAIR on 24 June 1997. This animal remained negative for more than 100 days post-inoculation (PI).

13. Establishment of *P. vivax* Salvador I (PvSal I) strain in splenectomized and intact Aotus monkeys and extraction of *P. vivax* RNA for DNA cloning.

On 16 May 1997, one *P. falciparum* cured splenectomized Aotus was inoculated IV with 1.25 ml of frozen and washed Pv Sal 1 Aotus infected red cells. When parasitemia was near its peak 15 days after inoculation, four additional splenectomized monkeys were infected with 5 X 10^6 parasitized erythrocytes, IFA slides and cryopreserved blood were prepared at this time. Recipient monkeys were bled 10 days PI, 5 mls each and their blood transported the same day to NMRI in Rockville, MA, where RNA extraction was performed 14-16 hours thereafter.

On January 9, 1998 one intact *P. falciparum* cured Aotus was inoculated IV and IP with a frozen stock of *P. vivax* Sal 1 strain passaged in splenectomized animals. When the animal reached 4.3 x 10^6 parasites x ml on day 12 the parasite was further passaged into an intact *P. falciparum*

cured Aotus, this time the animal peak on day 8 PI with 4.3×10^6 parasites x ml. Further passages were done in four additional intact monkeys until the parasitemia peak and stabilized at around 20,000 parasites x *ul* on day 12 PI. Only one of six animals self-cured and the others, either had recrudesences or low grade parasitemias <10 parasites x *ul*.

14. Toxicity of an oral route of administration of WR255663AK (JN8331), Artelinic acid in Aotus.

Artelinic acid an Artemisinin derivative is known to posess in vitro and in vivo antimalarial activity against strains of *Plasmodium falciparum* and *Plasmodium berghei*. In order to test Artelinic acid toxicity by the oral route in an Aotus monkey-model, on 12 August 1997, one Aotus (weighing 983 grms) cured of malaria infection was administered 20 mg/kg of WR255663AK (JN83331) Artelinic Acid orally in 5% sodium carbonate pH 8.4, twice daily fo three consecutive days. During treatment the animal was monitored for weight loss, depression, anorexia, vomiting or neurological signs. Apart from a transient loss of 13% body weight, which was gradually recovered over a month period, no other side effects were observed during treatment and follow up.

15. Efficacy of an oral route of administration of WR255663AK (JN8331), Artelinic acid against a *P. falciparum* FVO strain infection in Aotus.

In a toxicity study shown above, an oral dose of 20 mg/kg of WR255663AK (JN8331) Artelinic acid administered orally, twice a day for three days proved to be safe when tested in Aotus. On 5 September 1997, one malaria naive Aotus (weighing 823 grams) which had been infected with 1 ml of frozen *P. falciparum* FVO strain IP was treated orally with 20 mg/kg of WR255663AK (JN8331) Artelinic acid in 5% sodium carbonate pH 8.4 for three consecutive days, beginning on the day when parasitemia reached 5,000 parasites per cmm. As shown in Table 7 and 8 parasitemia cleared three days after initiation of treatment, but a recrudecence occurred 31 days PI with a peak parasitemia of 289,000 parasites x cmm on day 38 PI when retreatment was initiated, this time at 40 mg/kg of WR255663AK (JN8331) Artelinic acid orally, twice daily for three consecutive days. Parasite clearance and cured occurred on day 42 PI, four days after initiation of treatment. The animal remained negative up to day 100 PI when the experiment was terminated.

16. Immunogenicity and Efficacy of a *P. falciparum* EBA-175, AMA-1 and MSP-1 DNA Vaccine alone or in combination in Aotus Monkeys.

Forty malaria naive Aotus were divided into five groups of eight monkeys each and immunized intradermally with AMA-1, EBA-175 and MSP-1 plasmid

DNA vaccines alone or in combination three times at monthly intervals and then boosted at six months, in order to determine its immunogenicity and efficacy.

Results of the first challenge for groups 1, 2 and 3, carried out on August 12, 1997 with 1 x 10^5 parasites of *P. falciparum* FVO, were considered invalid when groups 4 and 5 plus a naive control failed to developed infection 56 days after inoculation. To overcome the unexpected loss of infection in Groups 4 and 5, which might have been due to a die off of the parasite, as it is presumed from an observed delay in patency in groups 1, 2 and 3 as shown in Table 9. It was collectively decided to modify the challenge procedure in the following way: Media for inoculation was changed from chilled saline to RPMI and all procedures were carried out at room temperature. Groups 4 and 5 were then re-vaccinated on October 8, fifty six days after inoculation and rechallenged on 28 October, seventy seven days after the first challenge with 1 x 10^5 parasites of the FVO strain. This time as shown in table 9a, all animals in Groups 4 and 5 became parasitemic with no detectable differences in pre-patent period, day to peak parasitemia or day of initiation of treatment with mefloquine. Therefore the vaccine candidates did not have any demonstrable effect on the course of parasitemia in these animals.

17. Immunogenicity and Efficacy of a *P. falciparum* EBA-175, AMA-1, MSP-1 DNA Vaccine as a combination with or without Aotus Granulocyte-Macrofage-Colony-Stimulating Factor (aGM-CSF) in Aotus Monkeys.

Twelve malaria naive Aotus were divided into four groups of 3 monkeys each and immunized intradermally with a combination erythrocytic stage malaria plasmid DNA vaccine consisting of EBA-175, MSP-1 and AMA-1 with or without co-delivery of an expression plasmid encoding an Aotus aGM-CSF, three times at monthly intervals and then boosted at six months, in order to test its immunogenicity and efficacy. Challenged with 1 x 10^5 parasites of a *P. falciparum* FVO strain was carried out on January 19, 1998. As Shown in Table 10 all animals were patent between days 6 and 7 PI. A naive control was treated with mefloquine on day 12 PI when reached 400,000 parasites x *ul*. On day 13 PI one animal from group 1, two animals from group 2, three from group 3 and two from group 4 were treated as well. Additionally, two animals from group one were treated on day 14 PI. Although, the remaining two animals, one from 4 and the other one from group 2, did not reach the 400,000 parasites x *ul* limit, both had to be treated on days 17 and 18 PI respectively, due to low hematocrit readings. Therefore, it could be concluded from this experiment that the candidate vaccines did not protect the monkeys against challenge.

18. Immunogenicity and efficacy of a *P. falciparum* EBA-175, AMA-1 and MSP-1 DNA vaccine as a combination in PfFVO single-cured Aotus monkeys.

Twelve single cured PfFVO Aotus monkeys were divided into two groups of six monkeys each and immunized intradermally three times at monthly intervals and then boosted at six months, in order to compare the immunogenicity and protective efficacy of a combination erythrocytic stage plasmid DNA malaria vaccine consisting of EBA-175, MSP-1 and AMA-1.

All animals were challenged with 1 x 10^5 parasites of a *P. falciparum* FVO strain on January 19, 1998. As shown on table 11 one animal from group 1 an another one from group 2, were first patent on day 8 PI, and both had to be treated due to low Htos on days 23-27 PI respectively. In addition, 3/6 monkeys from group 1 became patent between days 15-17 PI, of these, one was only transiently parasitemic between days 14-17 PI, with <10 parasites x *ul* of blood, and then recrudesce on days 30-36 remaining negative until day 56. Another one was also transiently parasitemic between days 14-17PI and then self-cured. The other one, had to be treated with mefloquine on day 24 PI due to a low Hto. The remaining two animals of this group remained negative for more than 56 days PI. In group two, 5/6 animals became positive between days 8-16, of these, one animal remained negative for more than 56 days PI and another one cleared its parasitemia on day 17 PI, but recrudesce between days 28-35 PI, self- curing on day 36 PI. The other four had to be treated with mefloquine between days 22-27 PI. One of these animals died of malaria related complications, even though its parasitemia was < 10 parasites x *ul* during the course of the experiment. Therefore, it is concluded that complete or partial protection was achieved in 4/6 (67%) monkeys from group 1 that received the triple combination vaccine, compared to only 2/6 (33%) in the control group.

19. Induction of immunity by repeated challenge with the FVO strain of *P. falciparum.*

Of the various *P. falciparum* strains adapted to non-human primates, the FVO (Vietnam-Oak Knoll) strain would be useful for vaccine studies as only 25-30% of infected Panamanian *Aotus* self-cure. The remainder of the infected animals require curative drug treatment or death will ensue. When evaluating a vaccine, the higher the proportion of self-cure, the greater the number of animals needed in each experimental group to assure that the animals are protected by the vaccine and not self-curing.

To compare the efficacy of an "artificial" vaccine with protection afforded by acquired immunity, an experiment was initiated to induce immunity by repeated trophozoite challenge. Initial results were given in the previous report. Briefly, malaria naive Panamanian *Aotus* were inoculated with 10,000 parasites of the FVO strain, the parasitemia monitored daily by

blood film examination, and the infection cured with mefloquine (40.0 mg/kg, oral) when parasitemia approximated 400,000 per cmm. About 4 to 6 weeks after infection cure, the animals will be rechallenged with parasites from a donor monkey whose infection was initiated by cryopreserved parasites. Donor animals, cured of infection, were recycled into the challenge group. Challenges were repeated until the monkeys demonstrated complete immunity as seen in Tables 6, 12.

20. Passage of a Chloroquine resistant AMRU-1 strain of *Plasmodium vivax* in *Aotus* monkeys.

On 29 October 1998, one *P. falciparum* cured *Aotus* was inoculated intraperitoneally (IP) with a frozen AMRU-1 strain of *P. vivax*. This animal was followed up with daily blood smears for evidence of parasitemia until it reached 4,870 parasites x *ul* on day 20 Post inoculation (PI) and then treated with 10 mg/kg of Chloroquine for five days. One ml of infected blood from this animal with less than 10 parasites x *ul* was collected and passaged into another *Aotus* on 4 December 1998, when its parasitemia reached 25,670 parasites x *ul* was treated with 10 mg/kg of Chloroquine for three days. Blood from this animal was freeze on day 19 PI when its parasitemia was 37,090 parasites x *ul*. Parasitemia remained high despite treatment and the animal self cured on day 36 PI. A third animal inoculated sequentially on 21 January, 1999 with frozen stock IP was positive on day 5 PI. This animal was used as donor for a drug evaluation study.

21. Reversal of chloroquine resistance with the co-administration of prochlorperazine (WR280001AC; BN 43106) and chloroquine (WR1544 BM;AR 20613) against infections of the AMRU-1 strain (CQR) of *Plasmodium vivax*.

Previous studies with a CQR *P. falciparum* have shown that it is possible to achieve *in vivo* reversal of CQR by the co-administration of prochlorperazine and chloroquine, as evidenced by infection cure. Neither drug alone effects such cure. In one study with the CQR AMRU-1 strain of *P. vivax*, data indicated that WR238605 (a primaquine analogue) administered at 1.0 mg/kg x 3, plus chloroquine (10.0 mg/kg x 3) cured 2 of 3 infections, WR238605, alone at this dose, clears parasitemia but with recrudescence. This study was designed to determine if CQR of the AMRU-1 strain can be reversed *in vivo* by prochlorperazine plus chloroquine. On 21 January, 1999 a donor *Aotus* monkey was inoculated with frozen stock of the AMRU-1 strain of *P. vivax*. Each of 7 *Aotus l. lemurinus*, cured of *P. falciparum,* males and females, were inoculated on 3 February, 1999 intravenously with 5 x 10^6 of *P. vivax* AMRU-1 strain parasites. Blood films were obtained on the day after inoculation and continued daily for the duration of the experiment. When parasitemias approximates 5,000 per

cmm, oral treatment for three days was initiated as follows: Group 1. Three monkeys received Prochlorperazine 20 mg/kg plus chloroquine 10 mg/kg x five days. Group 2. Three monkeys received Chloroquine 10.00 mg/kg x five days. Group 3. Untreated control. Infections were considered cured, when films remained negative for 100 days. Recrudescense will be treated on an ad hoc basis. As shown on Table 13-14, 2/3 monkeys from group 1 cleared parsitemias on the first day after treatment and remained negative for more than 16 days post-inoculation (PI), the day of this report. In group 2 and 3 all animals remained positive for more than 16 days PI.

22. Augmentation of PADRE 45 immunogenicity with CpG in *Aotus* monkeys.

This experiment was started in 05 May 1998 in order to determine the relative immunogenicity of a synthetic peptide derived from the PfCSP sequence (PADRE 45) with different CpG sequences, emulsified in Montanide and delivered IM to *Aotus* monkeys.

The rationale for this experiment was that CpG sequences (short synthetic DNA sequences modeled from bacterial DNA) will enhance the immunogenicity of PADRE 45 when delivered IM emulsified in Montanide ISA720 in *Aotus* monkeys.

Three groups of 3 animals each were injected unilaterally in the quadriceps (400 µl total volume). A total of 100 µg of PADRE 45 and 500 µg of one of three CpG sequences were injected per dose as follows: Group 1:PADRE 45 in Montanide 720 plus ODN 1968; Group 2: PADRE 45 in Montanide 720 plus ODN 2041; Group 3:PADRE 45 in Montanide 720 plus ODN 2006.

All animals were bled several times before and after immunization at two week intervals on 05 May, 25 May, 4 June, 15 June, 30 June, 14 July, 27 July, 11 August and 8 September and immunized three times, 05 May, 26 May and 16 June 1998. No challenge was carried out in this experiment. The animals receiving oligodeoxynucleotide containing either three of four CpG motifs produced antibodies that bound a recombinant CSP as measured in ELISA, and reacted with *P. falciparum* sporozoites as tested in a sporozoite immunofluorescent test. These responses were significantly greater than those seen in animals receiving the oligodeoxynucleotide withouth CpG motifs. These data indicate that oligodeoxynucleotides containing CpG motifs improve immunogenicity of peptide immunogens in non-human primates an may be immunopotentiators useful in humans.

23. Immunogenicity and Efficacy of a *P. falciparum* EBA-175, AMA-1, MSP-1 DNA Vaccine as a combination delivered intradermally with or without *Aotus* Granulocyte-Macrophage-Colony-Stimulating Factor (aGM-CSF) in *Aotus* Monkeys.

As shown on a previous experiment, twelve malaria naive Aotus immunized intradermally with a combination erythrocytic stage malaria plasmid DNA vaccine, consisting of EBA-175, MSP-1 and AMA-1 with or without co-delivery of an expression plasmid encoding an *Aotus* aGM-CSF, were not protected when challenged with 1 x 10^5 parasites of a *P. falciparum* FVO on January 19, 1998. Nine of the 12 originally recruited animals for this experiment were re-immunized on 1 December, 1998 and then re-challenged on 11 January, 1999 with 10,000 parasites of the FVO strain of *P. falciparum*. Sera were collected every two weeks beginning the day prior to the FVO infection and continuing every two weeks after infection. As shown on table 15 seven days after challenge a naive control became positive and was treated on day 12 PI when parasitemia reached 247,640 parasites x *ul*. One animal from group 2, another one from 4 and a re-challenge control animal became positive on day 10 PI, the rest except for two other animals became positive between days 12 and 14 PI. One animal from group 1 remained negative for more than 25 days. One animal from group 3 had a peak parasitemia of 1,210 parasites x *ul* and then self cured on day 23 PI. Another one from group 4 had a peak parasitemia of 1,040 parasites x ul self curing on day 18 PI. The rest had to be treated with mefloquine as follows: One animal from group 1 on day 20 PI due to a low hto reading. Two animals from group 2 on day 20 PI when they went over the 300,000 parasites threshold. One of these animals died malaria-associated causes despite being treated with mefloquine at 390,000 parasites/*ul*. One animal from group 3 was treated on day 21 and another one from group 4 on day 22 due to a low Hto reading. In conclusion only 1/2 animals from group 1 were protected from challenge in this experiment.

24. Immunogenicity and efficacy of a *P. falciparum* EBA-175 , AMA-1, MSP-1 DNA vaccine as a combination with or without aGM-CSF in *Aotus* monkeys immunized by the intramuscular route.

Aotus granulocyte-monocyte colony stimulating factor (aGM-CSF) is a cytokine that drives hemopoeitic stem cells to produce more cells of granulocytic and monocytic lineage. Previous studies have demonstrated a lack of immunogenicity of a DNA vaccine administered IM in *Aotus* monkeys. GM-CSF was incorporated into this multi-gene DNA vaccine protocol and administered IM to determine if GM-CSF can reverse the failure of the DNA vaccines alone to induce an effective immune response.

The objectives of this experiment was to compare the immunogenicity and protective efficacy of a combination erythrocytic stage malaria vaccine consisting of EBA-175, MSP-1, and AMA-1 with and without co-delivery of an expression plasmid encoding aGM-CSF when injected by the IM route.

The experiment consisted of two groups of six monkeys each which received: Group 1. AMA-1, EBA-175 and MSP-1 DNA vaccines IM and the 1012 vector without insert. Group 2 received plasmid backbones without insert plus aGM-CSF. Three naive animals served as non-vaccinated controls.

All animals were bled several times before and after immunization at two week intervals and immunized four times, 8 April, 01 June, 29 June and 1 September 1998. Challenge was carried out on 9 October, 1998 with 10,000 parasites IV of an FVO strain of *P. falciparum*.

As shown on table 16 all animals became patent by day 7 PI. Treatment with 40 mg/kg of mefloquine once, was initiated on day 11 PI in one animal from group 2 when it reached 400,000 parasites x *ul*. On day 12 PI, three animals from group 1 and three from group 2 including two naive controls had to be treated. On day 13 PI another naive control was treated. By day 18 PI one animal from group 2 was treated this time due to a low hto reading. Only one animal from group 1 selfcured on day 19 but recrudesce on day 42 PI (20 November, 1998) with a peak parasitemia on day 49 PI of 110,250 parasites x *ul* being treated on day 56 PI (December, 4 1998) due to a low hto reading. Serologicals results are pending. Two animals, one from group 1 and another one from group 2, died of unrelated causes before challenge. In conclusion no significant difference was observed between groups in this experiment.

25. Immunogenicity and Efficacy of *P. vivax* DNA vaccines based on PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP(regions II-IV) alone or in combination in *Aotus* Monkeys.

This experiment was started on 29 October 1997 in order to evaluate the immunogenicity of five components of a multi-component DNA vaccine against *P. vivax*, PvCSP, PvSSP2, PvMSP1(p42), PvMA1, PvDBP (regions II-IV) and to test the efficacy of the multi-component vaccine against a blood stage challenge. The experiment consisted of seven groups of monkeys. The first four groups (3 animals each) were immunized with a PvCSP (Group 1), PvSSP2 (Group 2), MSP-1(p42) (Group 3), AMA-1 (Group 4). The primary purpose of these four groups was to test immunogenicity of these four individual components. The final three groups included 8 monkeys each that were immunized with PvDBP (regions II-IV) (Group 5), a mixture of the five individual plasmids (Group 6), and a negative control plasmid (Group 7). These groups were evaluated for vaccine immunogenicity. Each monkey received 500ug/plasmid/dose, given intradermally at weeks 0, 4, 8, and 20. Challenge occurred on 27 April with 1 x 10^6 parasites of a *P. vivax* Sal-1

strain. As shown in table 17 thirty-five animals and two *P. vivax* naive controls were inoculated. One animal from group five died before inoculation due to unrelated causes. As shown on table 17, no significant differences were found between groups in regard to prepatent period, days to peak parasitemia, or self-cured rates.

The prechallenged IFA titers against sporozoites (spz) or infected erythrocytes (irbc) were as follows Group 1 (PvCSP) 1:5120 spz; Group 2 (PvSSP2) 1:320 spz; Group 3 (PvMSP1) 1:2560 irbc; Group 4 (PvAMA1) 1:1280 irbc; Group 5 (PvDBP) <1:10 irbc; Group 6 (5 gene mixture) 1:5120 spz, 1:320 irbc; Group 7 (negative control plasmid) <1:10 spz, <1:10 irbc. Following challenge there was a suggestion that the parasitemias in the monkeys immunized with PvMSP1 were lower than in other groups, however, this was not statistically significant in this experiment. The irbc IFAT titers following challenge were very high in all groups, suggesting that they may have been primed by cross reacting antigens from their previous exposure to *P. falciparum*.

26. Heterologous *Plasmodium falciparum* CAMP strain blood stage challenge of hyperimmune *Aotus* monkeys.

The objective of this experiment was to determine whether repeated challenge with one strain of *P. falciparum* induces immunity in *Aotus l. lemurinus* to blood stage challenge with a heterologous strain of *P. falciparum*, the CAMP strain. On 21 September 1998, eight *Aotus* monkeys that had already undergone seven previous *P. falciparum* FVO infections were challenged with 10,000 parasites of the CAMP strain, a strain of parasite originally isolated in Malaysia. Although FVO was isolated from Vietnam, genetic analysis shows that the two strains have a variety of allelic differences in the sequences of antigens of interest to vaccine developers. All animals were previously treated on 7 September with 50 mg quinine once a day for 5 days and 10 mg of Doxycycline once to eliminate any sub-patent FVO strain infections. Daily blood smears for parasite counting and blood dots on filter paper were taken for detection of any sub-patent FVO or CAMP infections using PCR directed against specific sequences in the genes encoding blood stage antigens. Sera were collected every two weeks beginning the day prior to the CAMP infection and continuing every two weeks after infection. Three *P. falciparum* naive controls were used. As shown on table 18 all became parasitemic by days 6 and 7 PI. Five hyperimmunized animals became parasitemic between days 7-9 PI. One became parasitemic on day 14 PI and the other two did no show evidence of parasites in their blood for more than 40 days PI. Control naive animals were treated with mefloquine 40 mg/kg on days 12 and 13 PI when they reached 400,000 parasites x ul. Parasitemias in the hyperimmune group ranged between <10 and 10,000 parasites x *ul* selfcuring between days 16-18 PI. No recrudescences were observed for 112 days PI. This

experiment concluded on 1/11/99 when the animals were considered cured. During this experiment it was observed that the prepatency period increases and the severity of infection decreases with each successive infection. After five infections 50% of the animals were immune; after six infections, all were immune. Subsequent challenges with blood stage parasites of a heterologous strain (CAMP) either failed to become parasitemic (2/8) or self-cured their infections (6/8). These findings indicate that a significant degree of strain-transcending immunity developed during the repetitive challenges with FVO, in spite of the measurable heterogeneity in the sequences of several parasite proteins of interest to malaria vaccine developers.

27. Immunogenicity and efficacy of a *P. falciparum* EBA-175, AMA-1 and MSP-1 DNA vaccine alone or in combination in *Aotus* Monkeys.

As shown on a previous experiment, Aotus immunized with AMA-1, EBA-175 and MSP-1 as a combination were not protected against a challenge with a *P. falciparum* FVO strain, all animals in Groups 4 and 5 became parasitemic with no detectable differences in prepatent period, days to peak parasitemia or day of intitiation of treatment. When these animals were re-challenged on 28 July 1998 with 10,000 parasites of a *P. falciparum* FVO strain as shown on Table 19, all animals became parasitemic, this time between days 10 and 11 PI. One naive control animal became parasitemic on day 6 PI and the other one on day 11 PI. One of these animals was treated on day 14 PI with mefloquine 40 mg/kg once. On day 16 PI one animal from group 4 was treatment with 10 mg/kg of Quinine for five days. Its parasitemia was suppressed for two days but went up to 533,990 parasites x ul on day 19 PI when it was decided to treat it with 40 mg/kg of mefloquine once due to an apparent resistance to quinine of the FVO strain. Quinine treatment was initiated in four animals from group 4 and two from group 5 on day 19 PI, but then were retreated with 40 mg/kg of mefloquine on day 20 PI because the animal that was first treated with quinine on day 16 PI died of malaria. Two other animals, one from group 4 and another one from group 5, were treated with mefloquine on day 21 PI. On day 22 PI five animals, two from group 4 and two from group 5 were treated. Of these, one animal from group 5 died despite treatment and another one that was treated the day previously died also. The second naive control was then treated with mefloquine due to a low hto reading. No significant differences were found between groups in regard to prepatent period, days to peak parasitemia, or day of treatment.

28. Adaptation of a Mefloquine resistant *P. falciparum* C2A clone to *Aotus* monkeys.

Mefloquine resistant strains of *P. falciparum* have been detected along the Cambodia-Thailand border in Asia. These strains have been studied *in*

vitro but until now adaptation to Aotus has been unsuccessful. The purpose of this experiment was to adapt Mefloquine clones to Aotus monkeys in order to do future drug resistant studies *in vivo*. On December 14, 1998 three splenectomized Aotus were inoculated Intravenously (IV) and IP with 1 and 3 mls respectively of cultured *P. falciparum* parasites strains WR75 and clones C2A and C2B brought from WRAIR. Seventy three days Post-Inoculation (PI) the C2A inoculated monkey (89005) became positive with a peak parasitemia of 10,500 x *ul* on day 84 PI selfcuring on day 106 PI. This animal died of cardiac arrest on day 124 PI. Blood from this animal was further passage six times into splenectomized an intact Aotus as shown in Table 20. An aliquoat of frozen stabilate was sent to WRAIR for further passage *in vitro* and for genetic analysis.

29. Reversal of Chloroquine resistance with the co-administration of Prochlorperazine (WR280001AC; BN 43106) and Chloroquine (WR1544 BM;AR 20613) against infections of the AMRU-1 strain (CQR) of *Plasmodium vivax*.

Previous studies with a CQR *P. falciparum* have shown that it is possible to achieve *in vivo* reversal of CQR by the co-administration of Prochlorperazine and Chloroquine, as evidenced by infection cure. Neither drug alone effects such cure. In one study with the CQR AMRU-1 strain of *P. vivax*, data indicated that Prochlorperazine administered at 20 mg/kg x 3 days in combination with Chloroquine at 10.0 mg/kg x 3 days cured 2 of 3 infections, whereas, Chloroquine alone at 10 mg/kg did not. This study was designed to repeat and reconfirm if CQR of the AMRU-1 strain can be reversed *in vivo* by Prochlorperazine plus Chloroquine.
On June 6, 1999 each of 10 *Aotus l. lemurinus*, cured of *P. falciparum,* males and females, were divided in four groups of three animals each and inoculated intravenously with 5 x 10^6 of *P. vivax* AMRU-1 strain parasites. When parasitemias approximated 5,000 per cmm, oral treatment was initiated for five days with the drugs alone or in combination as shown in Table 21-22. This time results demonstrated that 3/3 monkeys from group 3 cleared and cured parasitemias on the second day after treatment and remained negative for more than 44 days PI. This experiment re-confirmed reversal of chloroquine resistance of *P. vivax* AMRU-1 using the combination of Phroclorperazine plus Chloroquine.

30. Passage of the AMRU-1 strain (CQR) and the SAL-1 strains of *P. vivax* in Aotus for *in vitro* drug susceptibility testing and efficacy of Artelinic acid *in vivo*.

The emergence of Chloroquine resistant *P. vivax* is a newly emerging problem of antimalarial drug resistance. Since the first description of resistant *P. vivax* in Papua New Guinea, other resistant isolates have been

confirmed in Oceania, in Southeast Asia, and South America. Due to the difficulty of growing *P. vivax in vitro*, previous studies of drug resistance in *P. vivax* have been limited to clinical studies or with the one chloroquine resistant isolate that has been adapted to grow in Aotus monkeys. Therefore little work has been done to understand the underlying mechanism of resistance to chloroquine in *P. vivax.*

The purpose of this experiment was to expand upon the *in vivo* data obtained in previous experiment by taking *P. vivax* isolates from the monkeys and conducting resistance reversal studies *in vitro.*

On 24 June 1999, two Aotus cured of *P. falciparum* malaria infection were inoculated, intravenously with one ml of infected blood of the AMRU-1 and Sal-1 strains of *P. vivax*: Parasitemia were followed by daily blood smears and 1.5 ml of blood was collected aspetically once the peak parasitemia was reached for the *in vitro* studies.

Treatment was initiated on day 12 PI with 2 mg/kg of Artelinic Acid for three days. As shown in Tables 23-24 the AMRU-1 inoculated Aotus did not respond to treatment and remained positive up to 32 PI (18 days Post-Treatment). In contrast, the Sal-1 inoculated Aotus cleared parasitemias six days after finishing treatment and remained negative for more than 17 days.

31. Oral administration of Artelinic acid (WR 255663AK) against infections of *P. falciparum* FVO in Aotus monkeys.

The artemisinin antimalarial drugs generally are considered the most important class of drugs for the future control of infections due to multiple drug resistant *P. falciparum*. These drugs, originally isolated by Chinese scientists from sweet wormwood (*Artemisia annua*), have been used for the past decade in Asia and some other malaria endemic areas without the benefit of registration by drug regulatory authorities in the US or Europe. Artemisinin derivatives such as Artesunate, Artemether, and Dihydroartemisinin have been used primarily on the basis of limited preclinical data that is available on the class from the Chinese.

Although many of the preclinical efficacy studies have been completed for Artelinic acid, several important projects remain to be completed in Aotus monkeys infected with human malaria isolates. In this study we conducted a dose ranging study of Artelinic acid for the oral treatment of *P. falciparum* infections.

On July 7, 1999, each of ten malaria naïve Aotus were inoculated with 50×10^3 *P. falciparum* FVO malaria parasites IV and divided in five groups of two monkeys each.

As shown in tables 25-26, a supression on parasitemia was observed in 1/2 Aotus from group 1 on the second day of treatment. However, one animal died on day 5 after treatment and no effect was observed in the other one until it had to be treated at the next dose level of 8 mg/kg for three days

on day 14 PI. In the other groups parasitemia was cleared between days 1-4 after treatment. However all animals recrudesce between days 3-8 after treatment.

32. Efficacy of oral and intravenous administration of falcipain (APC3317) against infections of *P. falciparum* FVO in Aotus monkeys..

The cysteine protease falcipain is required for the degradation of hemoglobin by malaria parasites. Inhibitors of falcipain block hemoglobin degradation and development by erythrocytic parasites. The vinyl sulfone APC-3317 inhibits falcipain at low nanomolar concentrations. The compound also blocked the hydrolysis of hemoglobin and development of *P. falciparum* parasites in vitro and cured 40% of *Plasmodium vinckei*-infected mice. Primate studies are desired to test, for the first time, the efficacy of falcipain inhibitors against *P. falciparum* in vivo.

On February 4, 2000, each of 5 malaria-naïve *Aotus*, males and females, weighing from (811-1003) grms, were inoculated intravenously with 50 x 10^3 FVO *P. falciparum* and divided into two groups of two monkeys each and one control. As shown in Table 27-28 no effect of the drugs at 50 mg/kg by either of the two routes was observed over the parasitemia course. One animal from the intravenous group died during the injection on the first day of treatment due to toxic effects. The other one from this group died on the second day post treatment (PT). In the oral group one animal died on the third day PT. Neurological signs and cardiorespiratory arrest were observed before death in the IV group treated animals.

33. Oral administration of Artelinic acid (WR 255663AK) vs Artesunic Acid (BM 17174) against infections of *P. falciparum* FVO in Aotus monkeys.

On November 7, 1999 each of twenty four (24) malaria naïve Aotus were divided in two groups of twelve animals each and inoculated with 50 x 10^3 *P. falciparum* FVO malaria parasites IV and further divided into four groups of three monkeys each and treated with Artelinic Acid or Artesunic Acid as shown in Table 10.

Results of the experiment are summarized in Tables 29-32. Briefly, in the Artelinic Acid treated animals, all cleared parasitemias between days –1-1 after treatment. Recrudescence occurred in all between days 5-12 after finishing treatment and was dose dependent. Two animals from the Artelinic Acid treated group that received 32 mg/kg and one of them that was re-treated at 64 mg/kg died with signs of renal failure on days 26 and 21 PT respectively. Organs including kidneys will be send to WRAIR for pathology. On the Artesunic Acid treated animals, all cleared their parasitemias between –1-1 days after treatment. However, recrudescence occurred in all, between 6-14 days after treatment except for one animal of the 32 mg/kg group

which remained negative for 116 days, when the experiment finished.

34. Priming for *P. vivax* Antigens by Prior Infection with *P. falciparum* in *Aous* monkeys.

Aotus monkeys previously infected with *P. falciparum* (and cured) had greater immune responses to primary immunization with *P. vivax* antigens than is usually seen. This raises concerns that *P. vivax* antigens might not be best tested in monkeys that have a history of *P. falciparum* infection. The objective of this experiment was to determine whether prior exposure to blood stage infection with *P. falciparum* increases the immune response to subsequent primary immunization with *P. vivax* antigens.

On May 5, 1999 each of eight Aotus, four naïve and four previously exposed *to P falciparum* were infected with 10,000 parasites of the Sal-1 strain of *P. vivax* and divided in two groups of four monkeys each. As shown in Table 33, all animals were parasitemic between days 4 and 6 PI. Peak parasitemias were reached in Group 1 between days 13-14 with a minimum of 4.51×10^3 parasites x *ul* and a maximum of 78.53×10^3 parasites x *ul*. In Group 2 peak parasitemias were reached between days 14 and 18 with a minimum of 21.14×10^3 parasite x *ul* and a maximum of 72.48×10^3 parasites x *ul*. Only one animal from Group 1 had to be treated due to a low Hto reading. Parasitemias cleared in Group 1 (Previously exposed to *P. falciparum*) animals between days 27-37 PI. in contrast Group 2 animals (Naïve for malaria) cleared parasitemias between days 26-34 but two animals recrudesce on day 36 PI clearing between days 40-44 PI. No recrudescence was observed in group 1 animals after 64 days PI.

35. Passive transfer of anti-EBA-175 Region II protein monoclonal antibodies to *Aotus* monkeys infected with *Plasmodium falciparum.*

On 12 March, 1999, four monkeys were inoculated with 10,000 parasites of an FVO strain of *P. falciparum* in order to test if a Mouse monoclonal antibody directed against region II of EBA-175 from *P. falciparum* was able to provide protection to *Aotus* monkeys when infused IV during the early stages of a *P. falciparum* blood-stage infection. The experiment consisted of two groups of 4 monkeys each that on the last day of pre-patency received by an IV bolus, 4 mls of 15 mg/ml mouse monoclonal antibody in PBS. The same dose was administered again 24, 48 and 72 hours later for a total dose of 240 mg. The controls which consisted of 4 monkeys received by IV bolus 4 mls of 15 mg/ml of control mouse monoclonal antibody in PBS. The same dose was administered again 24, 48 and 72 hours later for a total dose of 240 mg. Results of this experiment are summarized in Table 34. Briefly, In group 1, 3/4 monkeys were treated with 40 mg/kg of Mefloquine once between days 13-15 PI either for high parasitemias > 400,000 parasites x *ul* or low htos, and only 1 animal with a

peak parasitemia of 57,380 parasites x *ul* selfcured on day 20 PI. In contrast, all group 2 animals were treated between days 14 and 17 PI due to parasitemias > 400,000 parasites x *ul.*

36. Immunization of Aotus monkeys against *P. falciparum* malaria with a plasmid encoding region II of EBA-175 followed with by a EBA-175 recombinant protein boost.

This experiment was started on 18 March, 1999 in order to determine if three immunizations with a plasmid encoding region II of EBA-175 followed by one immunization with EBA-175 region II recombinant protein produces protection from blood stage *P. falciparum* infection. The experiment consited of two groups of 6 monkeys and a third group of 3 monkeys. In group 1, all monkeys received three doses of a plasmid encoding EBA-175 (region II), and 500 *ug* of VR1721, a plasmid encoding *Aotus* GM-CSF, solubilized in PBS and delivered ID. Following the three doses of DNA vaccine, the animals received a boosting immunization consisting of baculovirus produced recombinant EBA-175 Region II protein emulsified in Montanide 720 containing 500 *ug* of CpG oligodeoxynucleotide 1968. The animals received half of protein dose SC along the flanks, and half IM in the quadruceps. Group 2 received three doses of one ml containing 500 *ug* of VR1050 the backbone plasmid of VR2527, and 500 *µg* of VR1721, a plasmid encoding *Aotus* GM-CSF, solubilized in PBS and delivered ID. These animals were then boosted with Montanide 720 and CpG (Adjuvant control), delivered both SC and IM as above. Group 3 was treated the same as Group 1 except that it received 100 *ug* of protein delivered IM only. Challenge with 10,000 parasites of a *P. falciparum* FVO strain was carried out on October 12, 1999. Results of this experiment are shown in Table 35. Briefly, on day five PI all monkeys became positive. The naïve control became positive on day 4 PI. Treatment with 20 mg/kg of mefloquine was initiated on day 11 PI in 3/6 monkeys from group 2 and 1/5 from group 1. Two out of three monkeys from group 3 were treated on this day also. By day 12 PI another monkey from group 2 and two monkeys from group 1 were treated. At that time the naïve control was also treated. The last monkey from group 2 was treated on day 15 PI. The remaining two monkeys from group 1 were treated on days 17 and 18 PI respectively, due to low htos readings. However, one of these monkeys died three days after treatment. Only 1 monkey from group 3 selfcured on day 25 PI and remained negative for the rest of the experiment.

37. Immune induction against Malaria infection in Aotus monkeys by topical ocular administration of a plasmid DNA vaccine encoding an AMA-1 *P. falciparum* blood stage antigen.

The ocular surface represents a unique milieu that is constantly exposed to toxic, antigenic and microbiological insults. In humans, the conjuctiva has been linked to an opened-up lymph node, with the exception that the antigens or infectious agents must transmigrate across the conjuntival epithelium before encountering the vast majority of immunocompetent cells within the substantia propia. Recently Plasmid DNA vaccines have been administered by the ocular route in mice, providing protection against a challenge with Herpes simplex virus. This hypothesized that Immunization of Aotus monkeys with a plasmid DNA vaccine directed against blood stage *P. falciparum* determinants by the ocular route will protect monkeys against a blood stage challenge. For this purpose on 18 March 1999, two naïve Aotus monkeys were immunized by the ocular route in both eyes with 50 *ul* of a dilution containing an AMA-1 plasmid vaccine three times at one month intervals. The animals were bled every two weeks and each time immediately before immunization. No seroconversion was observed in this experiment.

38. Effect of formulation in 150 mM Na phosphate buffer versus phosphate buffered saline on immunogenicity of DNA vaccines in Aotus monkeys.

Vival Inc has reported *in vivo* expression and improved immunogenicity of DNA vaccines formulated in Na phosphate as opposed to the standard formulation in phosphate buffered saline. The aim of this study was to confirm improved immunogenicity in primates in order to decide whether to formulate DNA vaccines in Na phosphate for planned human trials.

Each of 16 *P. falciparum* and *vivax* cured Aotus monkeys were divided in two groups of 8 monkeys each and immunized as follows: Group 1 received 500 ug/dose x 3 doses of VR2516 in PBS delivered ID to the lower back in six different sites. Group 2, received 500 ug/dose x 3 doses of VR2516 in 150 mM Na phosphate delivered ID to the lower back in six different sites. All animal received three doses of the plasmids at one month intervals. No challenge was carried out in this experiment. Results of this experiment are pending.

39. Adaptation of Mefloquine resistant *Plasmodium falciparum* strain 1088 and clone C2B to Aotus monkeys.

Mefloquine resistant strains of *P. falciparum* have been detected along the Cambodia-Thailand border with Asia. These strains have been studied *in vitro* but until now adaptation to Aotus have been partially successful, as indicated in a previous experiment when we successfully adapted the C2A clone of the WR75 strain of *P. falciparum* to Aotus. However all attempts to adapt strain 1088 and clone C2B have failed. The purpose of this experiment was to re-attempt the adaptation of strain 1088 and clone C2B of Mefloquine resistant *P. falciparum* to Panamanian Aotus. On 28th June,

2000 two splenectomized Aotus were inoculated intravenously with cultured *P. falciparum* Mefloquine resistant strain 1088 and clone C2B. These animals remained negative for 147 days post inoculation (PI). On July 10th two other splenectomized Aotus were inoculated with the same strains. This time the parasites were previously cultured *in vitro* using Aotus red cells. These animals remained negative for 138 days PI.

40. Efficacy and toxicity of the oral administration of Artelinic acid (WR 255663AK) vs Artesunic Acid (BM 17174) against infections of *P. falciparum* FVO in Aotus monkeys.

The artemisinin antimalarial drugs generally are considered the most important class of drugs for the future control of infections due to multiple drug resistant *Plasmodium falciparum*. These drugs, originally isolated by Chinese scientists from sweet wormwood (*Artemisia annua*), have been used for the past decade in Asia and some other malaria endemic areas without the benefit of registration by drug regulatory authorities in the US or Europe. Artemisinin derivatives such as artesunate, artemether, and dihydroartemisinin have been used primarily on the basis of limited preclinical data that is available on the class from the Chinese.

In this study we determined and compared curative doses of each drug, Artelinic Acid and Artesunate and demonstrated no renal toxicity. A secondary objective of this study was to identify an effective dose regimen in Aotus that can be used for the design of planned neurotoxicological studies in Rhesus monkeys (at AFRIMS).

Each of fourteen (14) malaria naïve Aotus were divided in two groups of six animals each and two controls. The animals were then inoculated with 50 x 10^3 *P. falciparum* FVO malaria parasites IV on March 21, 2000 and further divided into three groups of two monkeys each. Each group was treated orally once a day with Artelinic acid or Artesunic Acid for five days as shown in Tables 36-39. Animals were bled on the marginal ear vein daily for parasite determination by the Earle and Perez method and twice a week from the femoral vein for CBC, retyculocites count, Blood Urea Nitrogen (BUN) and creatinine determinations. Monkeys were also observed for signs of renal failure such as facial edema, weight lost and anorexia.

As shown on Table 36-37: Briefly, All animals in the Artelinic Acid group cleared their parasitemias on the fifth day of treatment, but recrudesce and were retreated as follows: Group 1 recrudesce on days 8-12 PT; Group 2 recrudesce on days 20-21 and Group 3 did not recrudesce until day 23 PT when all groups were retreated by mistake including group 3 which was still negative. The control group was treated when it reached more than 200,000 parasites x *ul* on days 9-11 PI and cleared on days 1 and 3 PT. Recrudesce occurred on days 25-27 PT and retreatment on day 29 PT. In the animals treated with Artesunic Acid as shown in Table 38-39 results were as follows: In group 1, all cleared on the fifth day of treatment and

recrudesce on days 9 and 20 PT. In group 2, clearance occurred on the fifth day of treatment and first day PT. Recrudesce occurred on day 9 and 21 PT. In group 3, both cleared on the fifth day of treatment. Retreatment occurred in all groups on day 21 PT eventhough group 3 animals were still negative. No renal toxicity was observed either by clinical signs or BUN and Creatinine determinations in any of the experimental animals. Pathological analysis of renal tissue from two animals that died on a previous experiment, failed to show drug induce renal failure.

41. Infection of Splenectomized and Intact *Aotus l. lemurinus* with a Novel Plasmodium.

On June 2, 2000 a splenectomized Aotus monkey was infected intravenously with a human frozen stabilate of a novel Plasmodium parasite isolated by Naval Medical Research Center investigators from humans in Guyana. This parasite has morphological characteristics that are inconsistent with the known species that normally infect human beings. Molecular analysis of the ribosomal RNA gene failed to distinguish the parasite from *P. vivax*. Follow-up of the inoculated Aotus with daily thick blood smears began the day after inoculation and continued for up to 159 days PI when the animal received a single dose of 20 mg/kg mefloquine orally. No parasites were detected during follow up.

42. Immunization with native and synthetic EBA-175 and $MSP1_{42}$ plasmids followed by recombinant protein boost

This experiment was started on September 5th, 2000 in order to determine if immunogenicity and protection can be improved by use of plasmids featuring mammalian rather than native codon usage, and whether the combination of $MSP1_{42}$ with EBA-175 in a protein boost schedule increases protection compared to EBA-175 against challenge *with P. falciparum* FVO. Previous experiments done in Lima, Peru indicated that native codon usage EBA-175 plasmid boosted by recombinant EBA-175, provides a degree of protection from elevated parasitemia and anemia after challenge with 10,000 *P. falciparum* (FVO) infected erythrocytes. Six groups of 6 Aotus each were immunized as follows: Group 1, received sD-RII =region II of EBA-175 (mammalian codon usage) in a VR1020 plasmid backbone alone with a boost of RecPichia-RII = Pichia-produced recombinant EBA-175 region II protein, emulsified in Montanide 720 and PBS (70:30) and containing CpG oligodeoxynucleotide 2006 (500ug/ml). Group 2, received sD-$MSP1_{42}$ = $MSP1_{42}$ (mammalian codon usage) in a VR1020 plasmid backbone alone follow by a RecBac-$MSP1_{42}$ = baculovirus-produced recombinant $MSP1_{42}$ protein, emulsified in Montanide 720 and PBS (70:30) and containing CpG oligodeoxynucleotide 2006 (500μg/ml). Group 3, received a combination of sD-RII and sD-$MSP1_{42}$ plasmids follow with a

recombinat protein boost of RecPichia-RII and RecBac-$MSP1_{42}$. Group 4, were immunized with DNA Control = VR1020 control plasmid lacking *P. falciparum* sequences and the Adj. Control = Montanide 720 and PBS (70:30) and containing CpG oligodeoxynucleotide 2006 (500ug/ml). Group 5, received RecBac-$MSP1_{42}$ on primary and booster immunization. Group 6 received de Adj. Control. Challenge was carried out on January 8th, 2001. During post-challenge follow-up, blood films for parasite enumeration, were performed daily and microhematocrits three times per week. Animals developing parasitemias >400,000 parasites/μl or experiencing a 50% decrease in hematocrit compared to pre-challenge baseline were treated with 20 mg/kg mefloquine orally as a single dose. Results of this experiment are shown on table 40. Briefly All monkeys became positive between days 5-6 PI. In group 1, 4/5 were treated on day 12 PI and one on day 17 PI. In group 2 all animals were treated between days 12-19 PI. Two of these animals died of malaria related complications on day 18 PI. In group 3, 5/6 animals were treated between days 13-17 PI one due to low Hto. The other one remained below the threshold and self control infection on day 24 PI remaining negative until day 30 PI when the experiment was completed. In group 4, all animals were treated between days 12-17 PI. One of these animals died of malaria related complications on day 18 PI. In group 5, 3/5 animals were treated between days 16-19 PI and one on day 20th due to low hto. The other two remained below the threshold for treatment for more than 23 days PI. In group 6, all animals were treated between days 13-15 PI. In conclusion, a delay on treatment was observed in 1/6 animals from group two and 1/6 from group five. Partial protection was achieved in 1/5 animals from group three, and in 3/6 (50%) from group five. Adding the delay on treatment (4 days difference with controls) in another animal from group five, it could be established that at least 5/11 (55%) animals that were immunized with plasmids and/or recombinant $MSP1_{42}$ were partially protected.

43. Obtaining *Plasmodium vivax* parasites and DNA required to sequence the *P. vivax* genome.

In order to begin the *P. vivax* genomic sequencing effort, a source of pure high molecular weight DNA was obtained from Aotus blood. Fourteen *Aotus l. lemurinus* monkeys were used in this experiment. Two monkeys were used to Passage the Sal-1 strain first and on December 26, 2000 the remaining twelve were infected by saphenous vein injection of 1×10^5 infected erythrocytes. Parasitemia was followed by daily thick films until it peaks in the range of 20-40,000/ ul. Isolation of Parasites and DNA: In brief, 3 ml blood was collected by femoral vein puncture. Pooled blood from the monkeys was passed over a leukocyte reduction filter and the leukocyte depleted erythrocytes washed in PBS. Lysis of erythrocytes was performed in dilute acetic acid, and the released parasites washed several times in PBS.

The purified parasites were then mixed with low melting point agarose and the agarose allowed to gel, so that parasites were embedded in the gel. The gel was then exposed to Sarkosyl and proteinase K at 56°C for 48 hours to digest parasite membranes and proteins and free the chromosomes within the gel. The gel was then stored in 50 mM EDTA until chromosomal DNA is required for library construction as part of the *P. vivax* genome project.

KEY RESEARCH ACCOMPLISHMENTS:

1. Absence or low antibody responses were confirmed for a PyCSP DNA vaccine by the IM route when Aotus were immunized with Hepatitis HsBAg DNA vaccine which is known to induce antibody levels in other primate species. A striking finding during the course of this experiment was that the co-administration of oligos, induced a high antibody response not previously seen when an equivalent dose of a PyCSP DNA vaccine was used.

2. Neither Aotus immunized intradermally with AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines alone or in combination, nor Aotus immunized intradermally with AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines in combination with or without Aotus Granulocyte-Macrofage-Colony-Stimulating Factor (aGM-CSF) were protected when challenged with an FVO strain of *P. falciparum.*

3. Low non protective antibody responses were observed in single cured *P. falciparum* Aotus monkeys immunized intradermally with *P. vivax* DNA vaccines based on PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP(regionsII-IV) alone or in combination and challenged with a P. vivax Sal-1 strain.

4. A C2A clone of a Mefloquine resistant *P. falciparum* strain was adapted to splenectomized *Aotus* after a 74 day prepatent period.

5. Chloroquine resistance reversal was achieved in *Aotus* infected with the AMRU-1 strain of *P. vivax* by using chloroquine at 10mg/kg and prochlorperazine at 20 mg/kg in combination.

6. Oligodeoxynucleotides (CpGs) when given intramuscularly to *Aotus* improved immunogenicity of a *P. falciparum* PADRE 45 peptide immunogen.

7. A significant degree of strain-transcending immunity developed in *Aotus* that were challenged repeatedily with an FVO strain of *P. falciparum* and then infected with a heterologous CAMP strain of *P. falciparum*.

8. Immunization with a plasmid encoding region II of EBA-175 followed with by a EBA-175 recombinant protein boost partially protected Aotus monkeys against *P. falciparum* malaria.

9. Both Artelinic Acid and Artesunic Acid at 8-32 mg/kg orally for five days were effective at clearing parasitemia in *P. falciparum* FVO inoculated Aotus without renal toxicity.

10. *Aotus* immunized with plasmids and/or recombinant $MSP1_{42}$ were partially protected against *a P. falciparum* FVO challenge.

REPORTABLE OUTCOMES:

I. Manuscripts:

Jones TR, Obaldia NIII, Gramzinski RA, Hoffman SL. 2000. Repeated Infection of *Aotus* Monkeys with *P. falciparum* Induces Protection Against Subsequent Challenge with Homologous and Heterologous strains of Parasite. Am J Trop Med Hyg. In Press

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Sim KL, Narum DL, Liang H, Fuhrmann SR, Obaldia NIII, Gramzinski R, Aguiar J, Haynes DJ, Moch K, and Hoffman SL. 2000. *Plasmodium falciparum* EBA-175 Region II DNA Vaccination Induces Biologically Active Antibodies Infection and Immunity. In Press.

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II. Presentations:

Ohrt C,....Obaldia N..... Status of Artelinic Acid development. 49th Annual Meeting of The American Society for Tropical Medicine and Hygiene. Westin Galleria & Oaks, Houston, Texas. October 29-November 2, 2000.

Jones TR, Gozalo AS, Obaldia N. et al. Anemia in Aotus Monkeys Infected with *P. falciparum*. 49th Annual Meeting of The American Society for Tropical Medicine and Hygiene. Westin Galleria & Oaks, Houston, Texas. October29-November 2, 2000

Jones TR, Obaldia NIII, Gramzinski RA, Hoffman SL. Repeated Infection of *Aotus* Monkeys with *Plasmodium falciparum* Induces Protection Against Subsequent Challenge with Homologous and Heterologous strains of Parasite. Am J Trop Med Hyg. Presented at the American Society of Tropical Medicine and Hygiene Meeting. Washington DC, November 28-December 2 1999

Obaldia NIII, Jones TR, Gramzinski RA, Charoenvit Y, Kolodny N, Kitov S, Davis HL, Krieg AM, Hoffman SL. Synthetic Oligodeoxynucleotides Containing CpG Motifs Enhance Immunogenicity of a Peptide Malaria Vaccine in Aotus Monkeys. Presented at the American Society of Tropical Medicine and Hygiene Meeting. Washington DC, November 28-December 2, 1999

Gramzinski RA, Kumar S, Aguiar J, Liang H, Sim BK, Obaldia N, Haynes D, Hobart P, Hoffman SL.: Immunogenicity and Protective Efficacy of a Plasmodium falciparum MSP-1, AMA-1 or EBA-175 DNA Vaccine Alone or in Combination in Aotus Monkeys. Presented at the American Society of Tropical Medicine and Hygiene Meeting. Lake Buena Vista, Orlando Florida

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Obaldia N, Gramzinski RA, Rossan RN, Collins WE, Oliveira D, Lal A, Hoffman SL.: Panamanian Aotus lemurinus lemurinus Susceptibility to Sporozoite Plasmodium falciparum Infection: A P. falciparum Challenge Model. Presented at the American Society of Tropical Medicine and Hygiene Meeting. Lake Buena Vista, Orlando Florida December 7-11, 1997.

Gramzinski RA., Maris DC, Obaldia N, Rossan R, Sedegah M, Wang R, Hobart P, Margalith M, Hoffman SL.: Optimization of Immune Responses to a Plasmodium DNA Vaccine in Aotus Monkeys. Presented at the American Society of Tropical Medicine and Hygiene Meeting. San Antonio, Texas. 17-21 November, 1995.

CONCLUSIONS:

1. Results of the challenge experiments of *Aotus* vaccinated with plasmid DNA vaccines coding for the AMA-1 and EBA-175 genes, showed that 1/3 monkeys were partially protected and self cured against challenge of *P. falciparum* (Vietnam-Oak Knoll strain).

2. Results of the inoculation of Panamanian *Aotus* vaccinated with a pre-erytrocytic plasmid DNA vaccine containing CSP, SSP2 and Exp-1 genes of *P. falciparum*, with sporozoites of a the Santa Lucia were inconclusive.

3. The absence or low antibody responses observed in previous experiments with a PyCSP DNA vaccine when *Aotus* were vaccinated by the IM route was confirmed when a distinct antigenic DNA vaccine as a Hepatitis HsBAg, know to induce antibody levels in other primate species was used. A striking finding during the course of this experiment was that the co-administration of oligos, induced a high antibody response not previously seen when an equivalent dose of a PyCSP DNA vaccine was used.

4. A frozen *Plasmodium falciparum* strain 1088 did not adapt when inoculated in Panamanian *A. l. lemurinus* monkeys by the IP route.

5. A Salvador I (PvSal I) strain of *P. vivax* was successfully adapted in splenectomized and intact *A. l. lemurinus* monkeys after serial in *vivo passage*.

6. Artelinic acid WR255663AK (JN8331) when given to Aotus monkeys by the oral route at 20 mg/kg twice daily for three consecutive days, appeared to be safe, and cleared a *P. falciparum* FVO infection three days after initiation of treatment. Re-treatment at 40 mg/kg cured a recrudecence that occurred 31 days PI.

7. Neither Aotus immunized intradermally with AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines alone or in combination, nor Aotus immunized intradermally with AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines in combination with or without Aotus Granulocyte-Macrofage-Colony-Stimulating Factor (aGM-CSF) were protected when challenged with an FVO strain of *P. falciparum.*

8. AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines when given intradermally as a combination to *P. falciparum* FVO cured Aotus protected 4/6 (67%) monkeys against an homologus re-challenge, in contrast to 2/6 (33%) in the control group.

9. Low non protective antibody responses were observed in single cured *P. falciparum* Aotus monkeys immunized intradermally with *P. vivax* DNA vaccines based on PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP(regionsII-IV) alone or in combination and challenged with a P. vivax Sal-1 strain.

10. The AMRU-1 strain of *P. vivax* reverted to chloroquine resistance (CQR) when selectively passaged and treated with chloroquine at 10 mg/kg for five days in *Aotus* monkeys.

11. A C2A clone of a Mefloquine resistant *P. falciparum* strain was adapted to splenectomized *Aotus* after a 74 day prepatent period.

12. Chloroquine resistance reversal was achieved in *Aotus* infected with the AMRU-1 strain of *P. vivax* by using chloroquine at 10mg/kg and prochlorperazine at 20 mg/kg in combination.

13. Oligodeoxynucleotides (CpGs) when given intramuscularly to *Aotus* improved immunogenicity of a *P. falciparum* PADRE 45 peptide immunogen.

14. Reimmunization and rechallenge with a *P. falciparum FVO* strain partially protected 1/2 *Aotus* that received an EBA-175, AMA-1, MSP-1 DNA vaccine as a combination with aGM-CSF intradermally.

15. *Aotus* immunized intramuscularlly with EBA-175, AMA-1, MSP-1 DNA vaccine as a combination with aGM-CSF were not protected against *a P. falciparum* FVO challenge.

16. A significant degree of strain-transcending immunity developed in *Aotus* that were challenged repeatedily with an FVO strain of *P. falciparum* and then infected with a heterologous CAMP strain of *P. falciparum*.

17. *Aotus* that were immunized with an EBA-175, AMA-1 and MSP-1 DNA vaccine intradermally as a combination were not protected when rechallenged with an FVO strain of *P. falciparum*.

18. A C2A clone of a Mefloquine resistant *Plasmodium falciparum* strain was adapted to splenectomized and intact *Aotus*.

19. Artelinic Acid (WR255663AK;BM04131) when given orally at 2 mg/kg x three days suppressed infections of the AMRU-1 (CQR) but cleared SAL-1 strains of *P. vivax* in Aotus monkeys.

20. Artelinic Acid (WR255663AK;BM04131) administered orally at 2-24 mg/kg x three days was effective against infections of *P. falciparum* FVO strain in Aotus monkeys.

21. Orally or intravenously administered falcipain (APC3317) was ineffective against infections of *P. falciparum FVO*.

22. Artelinic Acid and Artesunate were effective against infections with *P. falciparum* FVO in Aotus monkeys.

23. Passive transfer of anti-EBA-175 Region II protein monoclonal antibodies was not effective at controlling parasitemia in *Aotus* monkeys infected with *P. falciparum.*

24. Immunization with a plasmid encoding region II of EBA-175 followed with by a EBA-175 recombinant protein boost partially protected Aotus monkeys against *P. falciparum* malaria.

25. Topical ocular administration of a plasmid DNA vaccine encoding an AMA-1 *P. falciparum* blood stage antigen did not induce an immune response in Aotus monkeys.

26. Attempts to adapt a C2B and 1088 clone of a Mefloquine resistant *P. falciparum* strain to Aotus were unsuccessful.

27. Both Artelinic Acid and Artesunic Acid at 8-32 mg/kg orally for five days were effective at clearing parasitemia in *P. falciparum* FVO inoculated Aotus. without renal toxicity.

28. A Novel *Plasmodium vivax* like parasite from Guyana failed to infect a splenectomized Aotus monkey.

29. Aotus immunized with plasmids and/or recombinant $MSP1_{42}$ were partially protected against *a P. falciparum* FVO challenge.

30. *P. vivax* DNA was obtained for a genome sequence project.from infected Aotus blood.

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TABLE 1.

DETAILED ACTIVITY OF PROCHLORPERAZINE WR280003AC ALONE OR IN COMBINATION WITH WR 1544 BM CHLOROQUINE AGAINST INFECTIONS OF THE AMRU-1 STRAIN (CQR) OF *PLASMODIUM VIVAX* IN *AOTUS*.

AOTUS NO.	DAY PAT.	MG/KG DOSE	DAY PRE RX	PARASITEMIA PER cmm X 10^3 DAY OF TREATMENT 1	2	3	4	5	6	7	DAYS POST TREATMENT 1	2	3	4	DAYS NEG.
12651	6	*20 **10	5	14	18	4	0.28	0	0	0	0	0	0	0	6
12652	7	*20 **10	0.51	1.92	0.67	2.26	0.07	0.03	<10	0	0	0	0	0	5
12653	7	*20 **10	2.23	4.2	2	1.78	0.21	<10	0	0	0	0	0	0	6
12643	6	20*	18.1	16.9	35.4	32.8	27.7	25.8	19.9	15.43	15.7	26.1	37.5	32.3	0
12649	6	20*	9.2	18.4	22	24.6	40	15.7	12.6	18.4	15	29.6	8.9	16.9	0
12667	6	20*	3.8	6	16.9	30	8.1	4.9	5.9	9	6.9	12.3	1.8	7.6	0
12744	7	10**	1.1	0.22	<10	<10	0	0	0	0	0	0	0	0	8
12754	6	10**	3.8	12.8	35	17.9	20	0.91	0.12	0.11	<10	0	0	0	3
12755	6	10**	2.1	9.2	40.1	21.5	24.9	3.5	1.5	0.36	<10	0	0	0	3
12659 Control			6.1	20	21	23	5.6	1	0.82	0.22	<10	<10	0	0	2

*= Prochlorperazine
**= Chloroquine

TABLE 2

SUMMARY OF ACTIVITY OF WR280003AC (BN 43106) PROCHLORPERAZINE AND WR 1544 BM (AR 20613) CHLOROQUINE ALONE OR IN COMBINATION AGAINST INFECTIONS OF THE AMRU-1 STRAIN (CQR) OF *Plasmodium vivax* IN *AOTUS*

Monkey No.	Daily Dose x 7 Mg/Kg	Response of Parasitemia to RX			Days from initial Rx to parasite Clearance	Days from Final Rx to Recrudescence	Notes No of days negative
		None	Suppressed	Cleared			
12643	*20	+			37	n.a.	61
12649	*20	+			19	-	1, Died/anemia
12667	*20	+			18	4	74
12651	*20			+	4	n.a.	89
	**10						
12652	*20			+	7	n.a	85
	**10						
12653	*20			+	5	n.a.	87
	**10						
12744	**10			+	3	n.a.	88
12754	**10			+	8	n.a.	8, Died/anemia
12755	**10			+	8	n.a.	84

*Prochlorperazine
** Chloroquine

TABLE 3

DETAILED PARASITEMIA OF *AOTUS* MONKEYS VACCINATED WITH A PLASMID DNA AMA-1 VACCINE AND CHALLENGED WITH AN FVO STRAIN OF *PLASMODIUM FALCIPARUM*

Parasitemia x cmm

		PI/DAY														
MONKEY	GROUP	4	5	6	7	8	9	10	11/am	11/pm	12/am	12/pm	13/am	13/pm	14	15
12769	1	0	0	<10	<10	<10	960	30,800	190960	200200	246400	235620	*311080			
12770	1	0	0	0	<10	<10	58,520	40,040	106260	95080	141680	86240	80060	47700	100100	*385000
12787	1	<10	<10	<10	<10	780	17940	33880	111690	184800	289170	198040	251020	*297810		
12788	2	0	0	0	<10	<10	23100	42110	92400	113960	204820	194580	190960	203280	*291060	
12789	2	0	0	0	<10	1540	49480	72380	249480	223300	*301880					
12790	2	0	0	<10	<10	<10	23340	32340	141680	129360	158620	207900	261800	*353440		
12791	3	0	0	0	<10	<10	7700	27720	123200	100190	*310370					
12792	3	0	0	0	<10	<10	890	18550	117430	107280	*291820					
12793	3	0	0	0	<10	<10	30110	58610	214060	175560	263340	*321120				

PI/DAY = Post inoculation day
* = day of initiation of treatment with mefloquine
Parasitemia = parasites x ml of blood

TABLE 4

DETAILED PARASITEMIA OF *AOTUS* MONKEYS VACCINATED WITH A PLASMID DNA AMA-1 VACCINE AND RE-CHALLENGED WITH AN FVO STRAIN OF *PLASMODIUM FALCIPARUM*

Parasitemia x cmm

PI/DAY	7	8	9	10	11	12	13	14	15	16	17	18	19	20
NP15														
12770	<10	0	0	0	<10	<10	<10	<10	>10	1996	16940	8760	50620	5910
12787	0	<10	<10	<10	<10	<10	<10	<10	0	0	<10	<10	<10	<10
12789	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
12790	0	<10	0	0	0	0	0	0	<10	<10	<10	12910	24660	9240
12791	0	<10	0	0	0	0	0	0	<10	<10	<10	3620	16940	30800
12792	<10	<10	<10	<10	910	810	<10	<10	<10	<10	<10	<10	<10	0
12793	<10	<10	>10	<10	1420	16940	3000	370	530	12320	2180	30800	22710	86240

PI/DAY	21	22	23	24	25	26	27	28	29
12770	260	*<10							
12787	<10	>10	<10	<10	<10	<10	<10	<10	*<10
12789	<10	<10	<10	<10	<10	0	0	0	0
12790	94800	16940	7840	*1580					
12791	9100	27720	10780	*2050					
12792	0	*<10							
12793	4280	<10	<10	*<10					

PI/DAY = Post inoculation day
* = day of initiation of treatment with mefloquine
Parasitemia = parasites x ml of blood

TABLE 5

DETAILED PARASITEMIA OF *AOTUS* MONKEYS VACCINATED WITH A PLASMID DNA EBA-175 VACCINE AND CHALLENGED WITH AN FVO STRAIN OF *PLASMODIUM FALCIPARUM*

	Parasitemia x cmm															
PI/DAY	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
12806	<10	<10	<10	38500	23100	249000	170090	273360	*591360							
12807	<10	<10	<10	55440	93940	*449680										
12808	<10	<10	<10	70840	27720	*492800										
12809	<10	<10	<10	45610	32410	*344960										
12810	<10	<10	<10	26180	19010	*312210										
12811	<10	<10	<10	27760	29260	285000	242680	281080	191120	176320	26170	12360	4010	360	<10	0
12812	<10	<10	<10	34800	19560	*431200										
12813	<10	<10	<10	32340	16920	172480	167800	259080	124740	175380	239090	123200	186350	267960	*189380	DIED
12814	<10	<10	<10	36960	9560	257920	229110	*517440								

PI/DAY = Post inoculation day
* = day of initiation of treatment with mefloquine
Parasitemia = parasites x ml of blood

TABLE 6

CHALLENGE WITH THE FVO STRAIN OF *PLASMODIUM FALCIPARUM*

MONK NO.	NO. OF CHALLENGES	NOTES
12727	6	Sterile immunity
12730	6	Sterile immunity
12735	6	Sterile immunity
12739	6	Sterile immunity
12762	5	Sterile immunity
12749	5	Sterile immunity
12748	4	Sterile immunity
12756	4	Sterile immunity
12757	4	Sterile immunity
12759	4	Sterile immunity
12763	4	Sterile immunity
12765	4	Sterile immunity
12752	4	Not immune/Died/49 days/PI
12764	3	Died Malaria/25 days/PI
12169	2	Died day 32 days/PI, malaria
12687	2	Rx,died day 46 days/PI, inter-current infection
12738	2	Died day 19/PI, malaria
12740	2	Rx,died 51 days/PI inter-current infection
12731	1	Died of Malaria 17 days/PI
12726	1	Died of Malaria 18 days/PI
12761	1	Died of intercurrent infection 46 days/PI
12768	1	Died lung aspiration17 days/PI
12786	2	Died/Malaria 23 days/PI

TABLE 7

DETAILED ACTIVITY OF ARTELINIC ACID (WR255663AK; JN8331) AGAINST INFECTIONS OF THE FVO STRAIN OF PLASMODIUM FALCIPARUM IN AOTUS

				PARASITEMIA PER CCMM X 10^3										
				DAY OF TREATMENT			DAYS POST TREATMENT							Days
MONKEY #	DAY PAT	MG/KG	DAY PRE	1	2	3	1	2	3	4	5	6	7	Neg.
12893	3	20	16.2	5.6	1.5	0.01	0	0	0	0	0	0	0	20
12893*	31	40	227.9	289.4	123.2	33.8	0.01	0	0	0	0	0	0	59

*=Retreatment

TABLE 8

SUMMARY OF ACTIVITY OF ARTELINIC ACID (WR255663AK; JN8331) AGAINST INFECTIONS OF THE FVO STRAIN OF PLASMODIUM FALCIPARUM IN AOTUS

MONKEY #	Daily Dose x 7 Mg/kg	Response of Parasitemia to Rx			Days from initial Rx to parasite Clearance	Days from final Rx to Recrudescence	Notes No. of days Neg.
		None	Suppressed	Cleared			
12893	20			X	3	21	21
12893*	40			X	4	0	59

*=Retreatment

TABLE 9

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 ALONE AND CHALLENGED WITH A P. falciparum FVO STRAIN

Parasites x cmm

PI/DAY

Monkey No.	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
12835	1	0	0	0	0	0	<10	<10	<10	49280	58520	221760	308000	314160	401090*		
12836	1	0	0	0	0	0	<10	<10	<10	18440	21560	105610	213000	272400	543920*		
12837	1	0	0	0	0	0	0	0	0	0	<10	<10	<10	3110	60160	97940	320400
12838	1	0	0	0	0	0	0	0	0	0	0	0	0	1060	980	36960	348110
12840	1	0	0	0	0	0	<10	<10	<10	<10	10780	140940	235500	326480	309940	408000	351120
12852	1	0	0	0	0	0	<10	<10	<10	15400	19890	117040	380000	430480	566720*		DIED
12841	1	0	0	0	0	0	0	0	<10	<10	7710	89970	106500	217920	469800*		
12844	1	0	0	0	0	0	0	0	<10	<10	3010	30010	99000	84160	289520	240960	394260*

Monkey No.	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
12877	2	0	0	0	0	0	0	0	0	<10	<10	2970	480	86390	47790	537440*	
12845	2	0	0	0	0	0	0	0	<10	<10	740	7690	2380	132420	314160	304960	763440*
12846	2	0	0	0	0	0	0	0	<10	<10	970	147860	109500	301840	283340	283940	409580*
12847	2	0	0	0	0	0	0	0	<10	<10	1010	66220	90000	331200	375760	334080	689920*
12848	2	0	0	0	0	0	0	0	<10	<10	490	47090	102000	194800	264880	341760	389520*
12860	2	0	0	0	0	0	0	0	<10	0	<10	<10	<10	<10	<10	21560	70010
12849	2	0	0	0	0	0	0	0	<10	<10	<10	4620	1180	151680	283360	482110*	
12851	2	0	0	0	0	0	0	0	0	0	0	0	0	<10	<10	1620	28820

Monkey No.	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
12850	3	0	0	0	0	0	0	0	0	0	0	0	<10	<10	1590	7700	95440
12855	3	0	0	0	0	0	0	0	0	0	0	0	0	<10	<10	13860	13860
12856	3	0	0	0	0	0	0	0	0	0	0	0	<10	<10	18460	50820	271040
12857	3	0	0	0	0	0	0	0	0	0	0	0	0	<10	1110	980	13010
12858	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12859	3	0	0	0	0	0	0	0	0	0	0	0	<10	<10	40610	47750	320370
12861	3	0	0	0	0	0	0	0	0	0	0	0	<10	<10	19850	33880	368010
12862	3	0	0	0	0	0	0	0	0	0	0	0	0	0	<10	<10	<10

* = Treatment with mefloquine

Group 1 = AMA-1

Group 2 = EBA-175

Group 3 = MSP-1

CONT... TABLE 9

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 ALONE AND CHALLENGED WITH A *P. falciparum* FVO STRAIN

Parasites x ccmm

PI/DAY

Monkey Number	Group	17	18	19	20	21	22	23	24	25	26
12835	1										
12836	1										
12837	1	431200*									
12838	1	581040*									
12840	1	501160*									
12852	1										
12841	1										
12844	1										

Monkey Number	Group	17	18	19	20	21	22	23	24	25	26
12877	2										
12845	2										
12846	2										
12847	2										
12848	2										
12860	2	338800	466720*								
12849	2										
12851	2	15460	361280	201000	528000*						

Monkey Number	Group	17	18	19	20	21	22	23	24	25	26
12850	3	110990	375760	288000	576000*						
12855	3	196340	369600	214500	326000	291500	576000*				
12856	3	437360*									
12857	3	78540	96400	125000	64500	73500	73500	127500	68750*		
12858	3	0	0	0	0	0	0	0	0	0	0
12859	3	399600*									
12861	3	560070*									
12862	3	920	123200	576000*							

* = Treatment with mefloquine

Group 1 = AMA-1

Group 2 = EBA-175

Group 3 = MSP-1

TABLE 9a

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 AS A COMBINATION AND CHALLENGED WITH A *P. falciparum* FVO STRAIN

Parasites x cmm
Day/PI

Monkey No.		6	7	8	9	10	11	12	13	14	15	16
12863	4	<10	<10	190	16820	66000	364500	812050*				
12865	4	0	<10	80	17760	43500	341250	1094900*				
12866	4	0	<10	<10	12900	38700	152250	466370*				
12869	4	0	<10	90	1980	19500	88500	372000	492000*			
12870	4	0	<10	240	8320	40500	141000	512560*				
12872	4	0	<10	90	2440	16500	67800	202500	246250	388010	197120	458560*
12873	4	0	<10	70	4370	18300	141750	594510*				
12875	4	0	<10	50	2780	5110	49920	355500	693750*			

Monkey No.		6	7	8	9	10	11	12	13	14	15	16
12879	5	0	<10	160	11360	81000	179250	477160*				
12822	5	0	0	<10	7650	16900	130500	339720	813400*			
12823	5	0	<10	<10	9550	28770	136500	586120*				
12829	5	0	0	<10	9350	47850	124450	421500*				
12830	5	0	0	<10	4820	20100	81750	354750	480750*			
12832	5	0	0	<10	1420	2520	62250	242250	310500	402000*		
12878	5	0	<10	180	3750	9080	76540	300750	1299280			

Monkey No.		6	7	8	9	10	11	12	13	14	15	16
12880	Control	<10	<10	330	29880	109500	407250*					
12896	Control	<10	<10	110	38000	99000	293250	655600*				
12897	Control	<10	<10	<10	1940	5830	31800	315750	389250	378640	369660	442080*
12898	Control	0	<10	100	3180	73500	106500	560250*				

*=Treatment with mefloquine
Group 4= Combination vaccine
Group 5= Plasmid control
Group Control= Malaria Naive

TABLE 10

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 AS A COMBINATION WITH OR WITHOUT aGM-CSF AND CHALLENGED WITH A *P. falciparum* FVO STRAIN

Parasites x cmm
DAY/PI

Monkey Number	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
12876	1	0	0	0	0	0	0	<10	>10	27940	8790	160160	220960	308000	382,240	366480*			
12882	1	0	0	0	0	0	0	<10	<10	6700	3080	157010	209420	332680	304,920	510000*			
12883	1	0	0	0	0	0	0	<10	<10	6158	21560	295680	226400	542080*					
12884	2	0	0	0	0	0	0	<10	>10	10780	7720	252160	277200	590910*					
12885	2	0	0	0	0	0	0	<10	>10	18480	9240	285610	303840	569930*					
12886	2	0	0	0	0	0	0	<10	>10	>10	1060	18690	23100	30800	47,250	25710	9240	30800	48750*
12887	3	0	0	0	0	0	0	<10	<10	>10	20020	135520	166320	539110*					
12888	3	0	0	0	0	0	0	<10	<10	>10	980	135520	158020	401220*					
12890	3	0	0	0	0	0	0	<10	<10	>10	4620	115760	120190	576720*					
12889	4	0	0	0	0	0	0	<10	<10	>10	13960	224800	78540	331010	243000	384010	258720	314160*	
12891	4	0	0	0	0	0	<10	<10	>10	27720	13860	246400	120190	517440					
12892	4	0	0	0	0	0	0	<10	<10	>10	710	151720	136280	325520	408000*				
12901	CONTROL	0	0	0	0	0	<10	<10	>10	9240	6060	207120	470400*						

*= Treatment with mefloquine
Group 1= Triple combination without aGM-CSF
Group 2= Triple combination with aGM-CSF
Group 3= Plasmid control with aGM-CSF
Group 4= Plasmid control without aGM-CSF

TABLE 11

DETAILED PARASITEMIA OF *P. falciparum* FVO CURED AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 AS A COMBINATION AND RE-CHALLENGED WITH AN HOMOLOGUS STRAIN

Parasites x cmm

Day/PI

Monkey No.	Group	8	9	10	11	12	13	14	15	16	17	18
12771	1	0	0	0	0	0	0	0	<10	<10	<10	0
12772	1	<10	<10	<10	<10	<10	<10	>10	10780	283360	73920	127820
12773	1	0	0	0	0	0	0	0	0	0	0	0
12774	1	0	0	0	0	0	0	>10	3080	21560	27720	39910
12775	1	0	0	0	0	0	0	>10	860	1010	<10	0
12778	1	0	0	0	0	0	0	0	0	0	0	0
12779	2	<10	<10	<10	0	0	0	0	<10	<10	<10	<10
12781	2	0	0	0	0	0	0	>10	1510	1260	980	5970
12782	2	0	0	0	0	0	0	0	<10	<10	<10	0
12783	2	0	0	0	0	0	0	0	0	0	0	0
12784	2	0	0	0	0	0	0	0	0	<10	<10	0
12785	2	0	0	0	0	0	0	0	<10	<10	<10	<10

Monkey No.	Group	19	20	21	22	23	24	25	26	27	28	29	30-35
12771	1	0	0	0	0	0	0	0	0	0	0	0	<10
12772	1	124740	169400	82550	1893	308*							
12773	1	0	0	0	0	0	0	0	0	0	0	0	0
12774	1	27810	38990	20450	318	124	790*						
12775	1	0	0	0	0	0	0	0	0	0	0	0	0
12778	1	0	0	0	0	0	0	0	0	0	0	0	0
12779	2	<10	>10	1580	1497	1609	28750	59060	48810	184800*			
12781	2	7920	1908	5580	116	690*							
12782	2	0	0	0	0	0	0	0	0	0	<10	<10	<10
12783	2	0	0	0	0	0	0	0	0	0	0	0	0
12784	2	0	0	<10	>10*	<10	0	DIED/malaria					
12785	2	<10	<10	10	390	<10	0	0*					

Group 1= AMA-1, EBA-175, MSP-1

Group 2= Plasmid control

*=Treated with mefloquine

TABLE 12

CHALLENGE WITH THE FVO STRAIN OF *PLASMODIUM FALCIPARUM*

MONK NO.	NO. OF CHALLENGES	NOTES
12730	6	Sterile immunity
12735	6	Sterile immunity
12739	6	Sterile immunity
12749	6	Sterile immunity
12756	6	Sterile immunity
12757	6	Sterile immunity
12759	6	Sterile immunity
12763	6	Sterile immunity
12765	6	Sterile immunity
12762	5	Sterile immunity
12727	6	Sterile imm./died pneumonia
12748	4	Sterile imm./died interc. infect.
12752	4	Not immune/Died/49 days/PI
12794	4	Sterile immunity
12821	4	Not immune
12764	3	Died Malaria/25 days/PI
12169	2	Died day 32 days/PI, malaria
12687	2	Rx,died day 46 days/PI, inter-current infection
12738	2	Died day 19/PI, malaria
12740	2	Rx,died 51 days/PI inter-current infection
12731	1	Died of Malaria 17 days/PI
12726	1	Died of Malaria 18 days/PI
12761	1	Died of intercurrent infection 46 days/PI
12768	1	Died lung aspiration17 days/PI
12786	2	Died/Malaria 23 days/PI

TABLE 13

DETAILED ACTIVITY OF PROCHLORPERAZINE* (WR280001AC;BN43106) AND CHLOROQUINE** (WR 1544BM,AR20613) AGAINST INFECTIONS OF THE AMRU-1 STRAIN (CQR) OF *Plasmodium vivax* in Aotus monkeys.

				PARASITEMIA PER CMM X 10^3										
		RX INITIATED		DAY PRE .	DAY OF RX					DAY POST RX				Days
MONKEY #	DAY P.I.	DAY PAT.	MG/KG	RX	1	2	3	4	5	1	2	3	4	Neg.
12894	5	1	20* 10**	1.9	7.5	21.7	4.5	0.01	0.01	0	0	0	0	16
12900	5	1	20* 10**	1.7	10.5	26.2	2	0.01	0.01	0	0	0	0	16
12940	5	1	20* 10**	1.5	9	15.7	33.2	46.8	34.7	12	7.5	6	2.9	
12914	5	1	10**	0.76	4.09	8.4	12	22.5	7.09	6	1.6	4.5	1.3	
12911	5	1	10**	0.01	2.9	0.65	19.6	7.5	22.65	39.2	9	28.6	1.5	
12906	5	1	10**	1.04	6	36.7	15.1	14.8	6.04	2.9	1.7	3.9	8	
12910	5	1	CONTROL	1.06	5.7	19.5	45.1	48.3	48.5	24.1	25.8	24.1	24.1	
12943	5	1	CONTROL	0.47	4.9	17.4	16.5	66.4	60.4	60.4	49.8	40.7	43.7	

TABLE 14

SUMMARY OF ACTIVITY OF PROCHLORPERAZINE* (WR280001AC;BN43106) AND CHLOROQUINE** (WR 1544BM,AR20 AGAINST INFECTIONS OF THE AMRU-1 STRAIN (CQR) OF *Plasmodium vivax* in Aotus monkeys.

MONKEY #	Daily Dose x 5 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudes-cence	Notes No. of days negative
		None	Suppressed	Cleared			
12894	20* 10**			X	1		16
12900	20* 10**			X	1		16
12940	20* 10**	X					
12914	10**	X					
12911	10**	X					
12906	10**	X					
12910	CONTROL	X					
12943	CONTROL	X					

TABLE 15

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 INTRADERMALLY AS A COMBINATION WITH OR WITHOUT aGM-CSF AND RECHALLENGE WITH A *P. falciparum* FVO STRAIN

Parasites x cmm

MONKEY DAY/PI	GROUP	DAY/PI 1	2	3	4	6	7	8	9	10	11	12	13
12876	1	0	0	0	0	0	0	0	0	0	0	0	<10
12882	1	0	0	0	0	0	0	0	0	0	0	0	0
12884	2	0	0	0	0	0	0	0	0	<10	0	<10	<10
12885	2	0	0	0	0	0	0	0	0	0	0	<10	<10
12888	3	0	0	0	0	0	0	0	0	0	0	0	0
12890	3	0	0	0	0	0	0	0	0	0	0	0	<10
12889	4	0	0	0	0	0	0	0	0	0	0	0	0
12891	4	0	0	0	0	0	0	0	0	0	0	0	0
12892	4	0	0	0	0	0	0	0	0	<10	0	<10	<10
12901	CONTROL	0	0	0	0	0	0	0	0	<10	<10	>10	1040
12935	NAIVE	0	0	0	0	0	<10	>10	310	45300	51090	247640*	

MONKEY DAY/PI	GROUP	14	15	16	17	18	19	20	21	22	23	24	25	26
12876	1	>10	26250	16620	37750	38010	11570	4530*						
12882	1	0	0	0	0	0	0	0	0	0	0	0	0	
12884	2	>10	8250	10570	107640	223480	202340	413090*						
12885	2	>10	80300	64930	163080	167610	289920	390990*	158690	DIED				
12888	3	0	<10	<10	>10	>10	410	1210	610	<10	<10	<10	<10	
12890	3	<10	5750	31710	110990	25670	178180	102680	138920*					
12889	4	<10	6290	9060	33220	48320	71090	83050	90600	67950*				
12891	4	0	<10	<10	1280	9060	8110	27180	16610	10570	3300	<10	<10*	
12892	4	<10	<10	0	<10	0	0	0	0	0	0	0	0	
12901	CONTROL	18010	94000	77010	271800	295390	407360*							
12935	NAIVE													

*treatment

TABLE 16

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH P. falciparum EBA-175, AMA-1, MSP-1
DNA VACCINES AS A COMBINATION WITH OR WITHOUT aGM-CSF BY THE INTRAMUSCULAR ROUTE.

Parasites x cmm
DAY/PI

MONKEY	GROUP	1	2	3	4	5	6	7	8	9	10	11
12921	2	0	0	0	0	0	0	<10	1180	30910	78540	133200
12920	1	0	0	0	0	0	0	<10	660	18490	63140	109340
12923	1	0	0	0	0	0	0	<10	510	21560	92400	99760
12922	2	0	0	0	0	0	0	<10	230	30800	83160	126320
12927	1	0	0	0	0	0	0	<10	640	49110	83160	144760
12926	2	0	0	0	0	0	0	<10	1580	78380	163240	400910*
12932	2	0	0	0	0	0	0	<10	420	15400	38500	52360
12931	1	0	0	0	0	0	0	<10	320	40020	70840	116390
12934	1	0	0	0	0	0	0	<10	760	23100	93480	130900
12933	2	0	0	0	0	0	0	<10	800	43120	81080	158420
12912	CONTROL	0	0	0	0	0	0	<10	780	23100	70500	58520
12913	CONTROL	0	0	0	0	0	0	<10	520	24090	51090	167860
12915	CONTROL	0	0	0	0	0	0	<10	760	33880	93410	101640

Treatment*

MONKEY		12	13	14	15	16	17	18	19	20	21	22
12921	2	411020*										
12920	1	400990*										
12923	1	139910	284010	111400	57290	3110	<10	<10	<10	0	0	0
12922	2	429000*										
12927	1	610990*										
12926	2											
12932	2	80900	69460	239720	119290	119090	61910	141370*				
12931	1	555680*										
12934	1	376560	199320	287500	440920*							
12933	2	641960*										
12912	CONTROL	429210*										
12913	CONTROL	517440*										
12915	CONTROL	344960	401120*									

* =Treatment

TABLE 17

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH *Plasmodium vivax* DNA VACCINES BASED ON PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP (regions II-IV) alone or in combination

Parasites x cmm

		PI/DAY												
MONKEY	GROUP	1	2	3	4	5	6	7	8	9	10	11	12	13
86016	1	0	<10	<10	<10	180	130	410	810	5010	19890	27110	20960	36820
87057	1	0	<10	<10	<10	260	1410	2120	880	15400	1090	8020	3960	6690
12791	1	<10	<10	<10	<10	1260	2640	6220	10720	24760	7610	3990	9950	2010
88039	2	0	<10	<10	<10	460	195	6890	11620	61600	46070	25520	20990	27110
86068	2	0	<10	<10	<10	140	100	350	<10	>10	320	980	490	<10
12790	2	0	<10	<10	<10	1340	2480	3080	5730	13860	1010	1120t		
88048	3	<10	<10	<10	<10	<10	120	<10	<10	>10	270	460	620	<10
12864	3	0	<10	<10	<10	380	590	1060	2130	18410	8910	12010	10680t	
12793	3	0	<10	<10	<10	710	1750	3490	3640	12330	12320	5810	590	<10
88047	4	0	<10	<10	<10	390	620	1850	1120	27790	13860	9240	8370	7940
12874	4	0	<10	<10	<10	490	1860	3990	1950	9280	26940	13810	5970t	
12792	4	<10	<10	<10	<10	660	1980	7010	12510	29280	33560	16540	7910	1940t
86019	5	0	<10	<10	<10	220	570	1040	1150	4620	1590	8640	1750	1920
12770	5	0	<10	<10	<10	890	6030	6010	15500	19960	8760	4510t		
12795	5	0	<10	<10	>10	520	1530	15510	21970	46200	46200	33040	18090	13560
12802	5	0	<10	<10	>10	610	3020	12940	24500	16940	10780	21010	12390	10110
12807	5	<10	<10	<10	>10	940	5970	13860	22500	86240	35420	27110	44660	29910
12810	DEAD													
12819	5	0	<10	<10	>10	920	1420	3930	8100	30800	8980	1160	560	<10
12676	5	0	<10	<10	>10	810	4960	8990	23840	36970	35420	25500	26180	8940
87024	6	0	<10	<10	<10	620	2110	1750	2010	13860	18090	10110	19770	12060
12787	6	0	<10	<10	<10	610	1020	1880	3740	1780	1500	810t		
12798	6	0	<10	<10	>10	400	2010	2010	6700	40040	21540	27000	13960	24090
12806	6	<10	<10	<10	>10	880	1670	7810	10870	56980	43120	24020	21560	27090
12808	6	0	<10	<10	>10	580	1350	3810	9210	55440	49280	34010	16940	20020
12812	6	0	<10	<10	>10	890	2210	4010	7120	43120	35510	13520	4970	3090
12820	6	0	<10	<10	<10	390	860	3080	1120	24110	21560	10020	11890	2960
11937	6	0	<10	<10	>10	890	1510	7560	15370	18490	3070	7740	5860	8920
88002	7	0	<10	<10	<10	980	1830	8240	15750	43120	50820	28510	22100	8970
12789	7	0	<10	<10	<10	740	2620	1970	1220	26740	6110	5890	6990	1100
12799	7	0	<10	<10	>10	1040	2110	14320	22840	73920	45330	39090	36960	18040
12809	7	0	<10	<10	>10	460	1690	3200	7500	78530	59060	38500	35420	42000
12811	7	0	<10	<10	>10	640	4860	5860	5620	47760	26180	3910	6910	4280
12814	7	0	<10	<10	>10	560	1980	2590	6500	18090	27320	19910	19840	9010
11928	7	0	<10	<10	>10	1420	4620	10500	16750	69300	41580	30090	33810	18110
11968	7	0	<10	<10	>10	1060	1720	3000	10870	21560	18480	13910	7890	19500t
12893	CONTROL	0	<10	<10	<10	370	710	2110	6450	26180	20010	11040	1050	1850
12895	CONTROL	<10	<10	<10	<10	780	1520	1330	5700	27720	27720	12910	30800	18020

t=treated *=Transfusion

TABLE 17 cont...

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH Plasmodium vivax DNA VACCINES BASED ON PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP (regions II-IV) alone or in combination

Parasites x cmm

		PI/DAY												
MONKEY	GROUP	14	15	16	17	18	19	20	21	22	23	24	25	26
86016	1	22590	13500	9290	3210	1210	660	<10	<10	0	0	0	0	0
87057	1	<10t												
12791	1	<10t												
88039	2	24390	18020	12320	4880	5110	3940	345	49	960	260	48	390	<10
86068	2	<10t				DIED								
12790	2													
88048	3	<10	<10	<10	<10	<10	<10	<10	0	0	<10	0	0	0
12864	3													
12793	3	<10	<10	0	0	0	0	0	0	0	0	0	0	0
88047	4	1780	2920	3080	660	<10	<10	<10	<10	<10	0	0	0	0
12874	4													
12792	4													
86019	5	<10	<10	<10	<10	0	0	0	0	0	0	0	0	0
12770	5													
12795	5	12500	2490	580	<10	<10	0	0	0	0	0	0	0	0
12802	5	1870	6910	1330	12890	5820	9260	330	460	<10	<10	<10	0	0
12807	5	16500	10500	12110	4420	3770	1720	1030	<10	<10	<10	<10	<10	<10
12810	DEAD													
12819	5	<10	<10	<10	0	0	0	0	0	0	0	0	0	0
12676	5	8010	610	<10	<10	0	0	0	0	0	0	0	0	0
87024	6	9970	1880	<10	<10	0	0	0	0	0	0	0	0	0
12787	6													
12798	6	2360	4660	3990	4810	1040	890	340	820	<10	<10	<10	0	0
12806	6	18090	3950	2950	3770	1010	<10	<10	<10	<10	<10	<10	<10	<10
12808	6	13590	11090	2050	1290	<10	0	<10	<10	<10	<10	<10	<10	0
12812	6	790t												
12820	6	10590	6890	2020	940	<10	0	<10	0	0	0	0	0	0
11937	6	5110	4010	7590	5620	2960	2990	2950	3180	1560	4660	4020	10090	4180
88002	7	3890	810	<10	<10	<10	0	0	0	0	0	0	0	0
12789	7	<10	<10	<10	<10t	0	0	0	0t					
12799	7	8910	7710	9020	1340	870	<10	<10	<10	<10	<10	<10	0	0
12809	7	21560	27410	10550	2220	4020	1390	163	380	1240	740	760	980	390
12811	7	880	1090	<10	<10	0	0	0	0	0	0	0	0	0
12814	7	2930	3560	1040	1170	<10	<10	<10	0	<10	<10	<10	0	0
11928	7	15920	8990	13500	3960	1890	980	380	<10	<10	<10	<10	<10	<10
11968	7										0	0	0	0
12893	CONTROL	<10	<10	<10	<10	0	0	0	0	0t				
12895	CONTROL	7930	870	1460	1910	590	0	<10	0t					

t=treated *=Transfusion

TABLE 17 cont...

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH Plasmodium vivax DNA VACCINES BASED ON PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP (regions II-IV) alone or in combination

Parasites x cmm

MONKEY	GROUP	PI/DAY 27	28	29
86016	1	0	0	0
87057	1			
12791	1			
88039	2	0	0	0
86068	2			
12790	2			
88048	3	0	0	0
12864	3			
12793	3	0	0	0
88047	4	0	0	0
12874	4			
12792	4			
86019	5	0	0	0
12770	5			
12795	5	0	0	0
12802	5	0	0	0
12807	5	<10	0	0
12810	DEAD			
12819	5	0	0	0
12676	5	0	0	0
87024	6	0	0	0
12787	6			
12798	6	0	0	0
12806	6	0	0	0
12808	6	0	0	0
12812	6			
12820	6	0	0	0
11937	6	2110t		
88002	7	0	0	0
12789	7			
12799	7	0	0	0
12809	7	290	<10	0
12811	7	0	0	0
12814	7	0	0	0
11928	7	0	0	0
11968	7	0	0	0
12893	CONTROL			
12895	CONTROL			

TABLE 18

DETAILED PARASITEMIA OF HETEROLOGOUS *Plasmodium falciparum* CAMP STRAIN
BLOOD STAGE CHALLENGE OF HYPERIMMUNE AOTUS MONKEYS

Parasites x cmm

MONKEY	DAY/PI 1	2	3	4	5	6	7	8	9	10	11	12	13
12749	0	0	0	0	0	0	0	0	0	0	0	0	0
12759	0	0	0	0	0	0	0	<10	590	870	1440	720	1220
12739	0	0	0	0	0	0	0	0	0	0	0	0	0
12756	0	0	0	0	0	0	0	0	<10	380	140	170	<10
12757	0	0	0	0	0	0	<10	<10	2980	960	1540	770	910
12765	0	0	0	0	0	0	0	<10	<10	1010	1100	1610	790
12763	0	0	0	0	0	0	<10	<10	670	1260	2300	2540	1250
12730	0	0	0	0	0	0	0	0	0	0	0	0	0
12910 control	0	0	0	0	0	<10	>10	1480	43120	53490	290250	484500*	
12911 control	0	0	0	0	0	<10	<10	>10	31600	42920	264750	519000*	
12943 control	0	0	0	0	0	0	<10	<10	12010	21560	66750	176250	767250*

MONKEY	14	15	16	17	18	19	20	21	22	23	24	25	26
12749	0	0	0	0	0	0	0	0	0	0	0	0	0
12759	2520	1340	560	<10	0	0	0	0	0	0	0	0	0
12739	0	0	0	0	0	0	0	0	0	0	0	0	0
12756	<10	<10	<10	<10	0	0	0	0	0	0	0	0	0
12757	<10	<10	<10	0	0	0	0	0	0	0	0	0	0
12765	9890	6710	750	<10	<10	0	0	0	0	0	0	0	0
12763	10920	5820	180	<10	<10	0	0	0	0	0	0	0	0
12730	>10	<10	<10	120	<10	0	0	0	0	0	0	0	0
12910 control													
12911 control													
12943 control													

* Treatment

TABLE 19

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 AS A COMBINATION AND RE-CHALLENGED WITH A *P. falciparum* FVO STRAIN

Parasites x cmm

DAY/PI

MONKEY	6	7	8	9	10	11	12	13	14	15	16	17
GROUP 4												
12863	0	0	0	0	0	<10	<10	>10	9090	15960	159020	197120
12865	0	0	0	0	0	<10	<10	>10	1620	13040	21100	72380
12866	0	0	0	0	0	<10	<10	<10	12110	31500	98060	324060
12869	0	0	0	0	0	<10	<10	<10	12990	12190	94510	56840
12870	0	0	0	0	<10	<10	<10	<10	15240	9560	84770	266010
12872	0	0	0	0	0	<10	<10	<10	1640	1980	65510	9010
12873	0	0	0	0	0	<10	<10	>10	181690	80060	663140t*	309540
12875	0	0	0	0	0	<10	<10	<10	1330	21000	126120	100100
GROUP 5												
12879	0	0	0	0	0	<10	<10	<10	5090	12160	50820	93940
12822	0	0	0	0	0	<10	<10	>10	480	9910	18010	51980
12823	0	0	0	0	0	<10	<10	>10	1310	4890	27000	9180
12829	0	0	0	0	0	<10	<10	>10	1540	18110	76090	78860
12832	0	0	0	0	0	<10	<10	<10	2050	16500	79260	15410
CONTROL												
12903	<10	<10	>10	660	640	77010	198030	246400	548910t			
12904	0	0	0	0	0	<10	<10	<10	2030	750	3090	2110

MONKEY	18	19	20	21	22
GROUP 4					
12863	306000	420910t*	216140m**		
12865	75590	379910	144090	116150	42750m**
12866	310900	528000t*	276320m**		
12869	86240	96040	47090	7440	1010m**
12870	369600	410050t*	114040m**		
12872	76560	91500	36960	74250m**	
12873	276000	533990m*	DIED		
12875	234080	422090t*	190200m**		
GROUP 5					
12879	92400	61500	15460	9360	1840m**
12822	344960	400110t*	297000m**		
12823	190810	63090t*	13960m**		
12829	149930	153000	120010	93620	DIEDm**
12832	278010	296010	324000	333710m*	DIED
CONTROL					
12903					
12904	1720	3010	980	1290	<10m**

*=Treatment **=Mefloquine

TABLE 20

Adaptation of *Plasmodium falciparum* C2A clone in Aotus monkeys

				PARASITEMIA X 10^3			TREATMENT									
PASSAGE LEVEL	MONKEY	DONOR MONKEY	DATE INOC	PREPATENT PERIOD	PEAK	DAY PI	DRUG	REGIMEN	DAY PI	RESULT OF TREATMENT	RECRUDESCE DAY/PI	RETREATMENT DRUG	RETREATMENT REGIMEN	RETREAT. RESULT	FOLLOW DAYS/PI	DISPOSITIO (day)
0	89005*	Culture	12/14/98	72	10.5	84	none	none	none	none	none	none	none	none	124	died(124)
1	92015*	89005	2/26/99	1	20.1	22	WR142490	40m/g/kg/3/days	73	cleared and cured	none	none	none	none	187	cured(93)
2	88011*	92015	3/15/99	0	12	6	none	none	none	none	none	none	none	none	15	died(15)
2	92034*	92015	3/15/99	0	85.1	11	WR142490	40 mg/kg once	11	suppresed	(44)(95)	none	none	none	221	self-cured(114
2	12971	92015	3/15/99	0	0.02	4	none	none	none	none	none	none	none	none	115	self-cured(15)
3	12987	92034	6/8/99	3	0.01	4	none	none	none	none	none	none	none	none	124	self-cured(4)
3	93014*	92034	8/12/99	2	121.5	8	WR255663	8.0 mg/kg/3/days	8	suppresed	16 36	WR255663 WR00297308	16MG/KG/3/days 20MG/KG/3/days	suppresse none	44	died (45)
3	12955	92034	8/12/99	2	1.7	11	none	none	none	none	none	none	none	none	124	self-cured(19)
4	12956	12955	8/25/99	2	0.38	7	none	none	none	none	none	none	none	none	111	self-cured(14)
5	12961	12956	9/4/99	11	0.96	28	none	none	none	none	none	none	none	none	101	self-cured(35)

*=Splenectomized

TABLE 21

DETAILED ACTIVITY OF PROCHLORPERAZINE* (WR280001AC;BN43106) AND CHLOROQUINE** (WR1544BM;AR20613) AGAINST INFECTIONS OF THE AMRU-1 STRAIN (CQR) OF *Plasmodium vivax* in Aotus Monkeys

	RX INITIATED			PARASITEMIA PER CMM X 10^3										
MONKEY #	DAY P.I.	DAY PAT.	MG/KG	DAY PRE .	DAY OF RX					DAY POST RX				
				RX	1	2	3	4	5	1	2	3	4	Days Neg.
12865	9	4	10**	1.8	7.5	11.4	45.3	31.71	54.3	27.1	31.7	44.3	36.2	none
12866	9	4	10**	0.86	4	2.59	2.06	0.89	0.01	0.01	0	0	0	3
12904	9	2	10**	0.76	2.2	8.94	13.01	10.57	6.08	8.8	8	3.53	5.01	none
12882	9	3	20*	0.62	1.89	5.02	12.96	12.08	24.9	25.4	11.7	2.8	0.56	none
12870	9	4	20*	0.58	2.91	5.12	25.67	19.63	29.6	22.65	8.1	6.04	1.34	none
12876	9	3	20*	1.36	6.04	11.7	24.1	27.18	64.9	19.91	8.8	8.81	1.26	none
12875	9	2	20*	0.71	5.12	5.1	6	0.21	0.01	0.01	0	0	0	116
12903	9	4	20* 10**	1.3	2.01	5.96	19.12	0.52	0.01	0.01	0	0	0	116
12880	9	2	20* 10**	0.28	2.86	2.6	13.5	1.32	0.01	0.01	0	0	0	81***
12869		4	control	0.74	3.02	3.91	10.57	22.5	25.6	25.6	15.1	7.55	4.5	none

*Phrochlorperazine 20 mg/kg
**Chloroquine 10 mg/kg
***=Out of experiment

TABLE 22

SUMMARY OF ACTIVITY OF PROCHLORPERAZINE* (WR280001AC;BN43106) AND CHLOROQUINE** (WR1544BM;AR20613) AGAINST INFECTIONS OF THE AMRU-1 STRAIN (CQR) OF *Plasmodium vivax* in Aotus Monkeys

MONKEY No.	Daily Dose x 5 mg/kg	Response of Parasitemia to Rx			Days from initital Rx to parasite Clearance	Days from final Rx to Recrudescence	Notes No. of Days Neg.
		None	Suppresed	Cleared			
12865	10**	X					
12866	10**	X					
12904	10**			X	6	4	3
12882	20*	X					
12870	20*	X					
12876	20*	X					
12875	20* 10**			X	6		116
12903	20* 10**			X	6		116
12880	20*			X	6		81***
12869	control						

*Phrochlorperazine 20 mg/kg
**Chloroquine 10 mg/kg
***=Out of experiment

TABLE 23

DETAILED ACTIVITY OF ARTELINIC ACID (WR255663AK;BM04131)
AGAINST INFECTIONS OF THE AMRU-1 (CQR)* AND SAL-1** STRAINS OF *Plasmodium vivax* in Aotus monkeys.

	RX INITIATED			PARASITEMIA PER CMM X 10^3													Days
				DAY PRE .	DAY OF RX			DAY POST RX									
MONKEY	DAY P.I.	DAY PAT.	MG/KG	RX	1	2	3	1	2	3	4	4	6	7	8	9	Neg.
12915*	12	12	2	32.71	39.4	26.6	10.5	6.63	1.99	1.59	2.06	2.7	0.88	1.86	1.42	0.82	87
12926**	12	12	2	24.66	19.12	19.71	5.19	2.76	0.98	0.44	0.01	0.01	0.01	0	0	0	94

TABLE 24

SUMMARY OF ACTIVITY OF ARTELINIC ACID (WR255663AK;BM04131)
AGAINST INFECTIONS OF THE AMRU-1* (CQR) AND SAL-1** STRAINS OF *Plasmodium vivax* in Aotus monkeys.

MONKEY #	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
		None	Suppressed	Cleared			
12915	2			X	18		87
12926	2			X	6		94

TABLE 25

DETAILED ACTIVITY OF ARTELINIC ACID (WR255663AK;BM04131)
AGAINST INFECTIONS OF *Plasmodium falciparum* FVO strain in Aotus monkeys.

				PARASITEMIA PER CMM X 10^3													
	RX INITIATED			DAY PRE .	DAY OF RX			DAY POST RX									Days
MONKEY	DAY P.I.	DAY PAT.	MG/KG	RX	1	2	3	1	2	3	4	5	6	7	8	9	Neg.
12982	7	4	2	1.2	51	5	0.01	0.01	0.01	0.01	0.01	0.01	DIED				0
12986	7	4	2	0.38	39.26	2.89	8.06	3.89	36.29	72.68	98.15	320.5***	191.7***	130.5***	4.99	2.66	0
12980	7	4	8	0.82	36.2	4.5	0.01	0	0	0	0	0	0	0	0.01	0.01	7
12981	7	4	8	0.34	24.16	2.99	0.01	0	0	0.01	0.01	0.01	0.02	12.08***	80***	2.8***	2
12988	7	4	16	0.36	43.7	17.8	2.1	1.01	0.01	0.01	0	0	0	0	0.01	0.02	4
12991	7	4	16	0.33	42.2	12.96	1.05	0.01	0.01	0.01	0	0	0	0	0.01	0.01	4
12985	7	4	24	0.89	34.7	10.5	0.01	0	0	0	0	0	0	0	0.01	0.01	7
12979	7	4	24	0.79	27.18	13.09	4.11	0.01	0.01	0.01	0	0	0	0	0.01	0.48	4
12990	11	8	4	0.29	200.17	96.64	49.83	19.6	40.1***	147***	6.04***	2.69	0.01	0	0	0	0
12989	10	7	4	0.39	289.76	87.58	39.26	63.42	85.5	86	320***	161***	40.5***	12.88	0.01	0.01	0

***=Retreatment at next dose level

TABLE 26

SUMMARY OF ACTIVITY OF ARTELINIC ACID (WR255663AK;BM04131)
AGAINST INFECTIONS OF *Plasmodium falciparum* FVO strain in Aotus monkeys.

MONKEY #	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
		None	Suppressed	Cleared			
12982	2		X				DIED
12986	2	X					
12980	8			X	1	8	7
12981	8			X	1	3	2
12988	16			X	4	5	4
12991	16			X	4	5	4
12985	24			X	1	8	7
12979	24			X	4	5	4
12990	4	X					
12989	4	X					

TABLE 27

DETAILED ACTIVITY OF ORALLY vs INTRAVENOUSLY ADMINISTERED FALCIPAIN (APC3317) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO STRAIN IN AOTUS MONKEYS

						PARASITEMIA PER cmm X 10^3 DAY OF RX										
			RX INITIATED		DAY PRE	1		2		3		DAY POST RX				Days
MONKEY #	ROUTE	DAY P.I.	DAY PAT.	MG/KG	RX	am	pm	am	pm	am	pm	1	2	3	4	Neg.
13001	Oral	8	8	50	8	6.1	33.2	129	83	138.9	343.9	273.6	579.8*		DIED	0
13000	Oral	8	8	50	6.8	9	39.2	143.4	18.1	73.9	214.4	143.6	675.5*			0
13002	IV	8	8	50	4.1	13.5	2.4 DIED									0
12972	IV	8	5	50	4	16.6	87	96.6	45.3	39.2	134.3	114.7	DIED			0
13004	None			None	2.8	4.5	31.7	63.4	16.9	113.2	374.4	168.9	525.3*			0

*=Treated with Mefloquine 20 mg/kg

TABLE 28

SUMMARY OF ACTIVITY OF ORALLY VS INTRAVENOUSLY ADMINISTERED FALCIPAIN (APC3317) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO strain in Aotus monkeys.

MONKEY #	Route	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
			None	Suppressed	Cleared			
13001	Oral	50	X					DIED
13000	Oral	50	X					
13002	IV	50	X					DIED
12972	IV	50	X					DIED
13004	None	50						

TABLE 29

DETAILED ACTIVITY OF ARTELINIC ACID* (WR 255663AK; BM04131) VS ARTESUNIC ACID (BM 17174) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO IN AOTUS MONKEYS

MONKEY	RX INITIATED			PARASITEMIA PER cmm X 10^3									
	DAY P.I.	DAY PAT.	mg/kg	DAY PRE	DAY OF RX			DAY POST RX					Days
				RX	1	2	3	1	2	3	4	5	Neg.
92031	8	3	32*	3.81	2.91	0.01	0	0	0	0	0	0	10
95007	8	4	32*	0.72	0.77	0.01	0	0	0	0	0	0	10
93020	8	3	32*	0.66	2.81	0.01	0	0	0	0	0	0	12
12994	8	4	24*	10.5	16.6	0.51	0.01	0	0	0	0	0	9
93031	8	4	24*	2.1	1.35	0.01	0	0	0	0	0	0	10
91009	8	4	24*	1.04	3.11	0.91	0.01	0	0	0	0	0	8
95001	8	4	16*	0.81	1.89	1.75	0.01	0	0	0	0	0	9
12996	8	4	16*	9	13.5	1.18	0.01	0	0	0	0	0	7
93017	8	4	16*	2.02	12	0.31	0.01	0	0	0	0	0	8
89061	8	4	8*	0.87	9.01	0.24	0.01	0	0	0	0	0	5
93034	8	4	8*	0.82	0.86	0.01	0	0	0	0	0	0.01	5
93033	8	2	8*	0.01	0.02	0.36	0.01	0	0	0	0	0.01	4
91020	CONTROL			0.51	0.67	45.12	31.5	192.7	504**	243**	65**	9.5	7
92019	CONTROL			0.98	1.09	33	9.18	150.9	410**	199**	63**	7.5	4
93030	CONTROL			0	0	0.01	0.01	0.01	9.04	19.6	116.2	24.2	14

**=Treatment Artelinic Acid 32 mg/kg

TABLE 30

DETAILED ACTIVITY OF ARTELINIC ACID (WR 255663AK; BM04131) VS ARTESUNIC ACID** (BM 17174) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO IN AOTUS MONKEYS

		RX INITIATED		DAY PRE	PARASITEMIA PER cmm X 10^3 DAY OF RX			DAY POST RX					Days
MONKEY	DAY P.I.	DAY PAT.	mg/kg	RX	1	2	3	1	2	3	4	5	Neg.
12995	8	4	32**	2.8	19.5	0.24	0.01	0	0	0	0	0	13
95020	8	3	32**	0.59	1.52	0.01	0	0	0	0	0	0	116
93026	8	3	32**	1.77	1.96	0.01	0	0	0	0	0	0	12
92004	8	4	24**	1.1	1.35	0.01	0	0	0	0	0	0	10
90034	8	4	24**	2.01	5.99	0.01	0	0	0	0	0	0	11
96025	8	4	24**	0.32	1.59	0.01	0	0	0	0	0	0	12
94014	8	4	16**	1.15	1.01	0.01	0	0	0	0	0	0	6
95011	8	4	16**	1.55	1.14	0.24	0.01	0	0	0	0	0	8
96021	8	4	16**	0.28	1.09	0.01	0	0	0	0	0	0	9
97003	8	4	8**	2.09	6.01	1.15	0.01	0	0	0	0	0	5
94011	8	4	8**	1.99	0.99	0.41	0.01	0	0	0	0	0	5
94006	8	4	8**	5.19	30.2	10.5	0.01	0	0	0	0	0	5

TABLE 31

SUMMARY OF ACTIVITY OF ARTELINIC ACID* (WR255663AK;BM04131) VS ARTESUNIC ACID (BM 17174) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO strain in Aotus monkeys.

MONKEY #	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
		None	Suppressed	Cleared			
92031	32*			X	-1	10	10
95007	32*			X	-1	10	10
93020	32*			X	-1	12	12(Died day 31 PI)
12994	24*			X	1	10	9
93031	24*			X	-1	10	10
91009	24*			X	1	9	8
95001	16*			X	1	10	9
12996	16*			X	1	8	7
93017	16*			X	1	9	8
89061	8*			X	1	6	5
93034	8*			X	-1	5	5
93033	8*			X	1	5	4
91020	32*			X	3	10	7
92019	32*			X	3	7	4
93030	32*			X	4	None	14 (Died day 35 PI)

*Retreatment was carried out at next highest dose

TABLE 32

SUMMARY OF ACTIVITY OF ARTELINIC ACID (WR255663AK;BM04131) VS ARTESUNIC ACID** (BM 17174) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO strain in Aotus monkeys.

MONKEY #	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx None	Suppressed	Cleared	Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
12995	32**			X	1	14	13
95020	32**			X	-1	None	116
93026	32**			X	-1	12	12
92004	24**			X	-1	10	10
90034	24**			X	-1	11	11
96025	24**			X	-1	12	12
94014	16**			X	-1	6	6
95011	16**			X	1	9	8
96021	16**			X	-1	9	9
97003	8**			X	1	6	5
94011	8**			X	1	6	5
94006	8**			X	1	6	5

Retreatment was carried out at next highest dose

TABLE 33

DETAILED PARASITEMIA OF AOTUS INFECTED WITH *Plasmodium vivax* SAL-1 STRAIN TO DETERMINE IF PRIOR EXPOSURE TO *Plasmodium falciparum* PRIME AOTUS TO *P. vivax* ANTIGENS

Parasitemia x ccmm x 10^3

MONKEY	GROUP	DAY/PI	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
12920	1		0	0	0	0	0	0	0.01	0.01	0.01	0.01	0.74	1.5	3.94	7.5	18.1	11.52
12921	1		0	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01	0.22	1.22	1.9	4.51	2.04	1.09
12922	1		0	0	0	0	0.01	0.01	0.01	0.01	1.01	1.81	6.51	15.1	29.75	57.38	78.52	49.35
12923	1		0	0	0	0	0	0.01	0.01	0.02	0.01	0.01	0.98	3.98	7.85	30.2	48.32	19.63
12973	2		0	0	0	0	0	0.01	0.01	0.01	0.02	0.8	2.54	3.9	11.52	34.73	72.48	40.77
12974	2		0	0	0	0	0.01	0.01	0.01	0.01	0.02	0.62	0.78	4.35	9.75	17.09	21.14	16.61
12977	2		0	0	0	0	0	0	0.01	0.01	0.02	0.64	1.78	2.54	8.82	22.14	23.09	24.16
12978	2		0	0	0	0	0	0	0.01	0.01	0.02	0.01	0.39	1.01	2.4	12.08	20.96	15.1

MONKEY	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
12920	16.72	19.5	12.98	9.06	3.05	2.42	0.68	0.01	0.01	0.01	0.01	0	0	0	0	0	0	0
12921	1.05	0.7	0.36	0.01**	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0
12922	27.18	32.25	27.18	6.01	1.89	1.12	2.18	0.01	0.01	0.02	0.98	0.59	0.49	0.29	0.62	0.36	0.01	0.01
12923	30.2	23.25	15.82	1.75	0.71	0.01	0.01	0.01	0.01	0.01	0	0	0	0	0	0	0	0
12973	36.24	39.26	21.14	6.89	12.08	10.02	6.4	2.11	0.94	0.86	0.01	0.01	0	0	0	0	0.01	0
12974	7.11	4.34	2.55	0.81	0.95	0.89	0.69	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0
12977	10.57	6.3	1.99	0.59	1.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
12978	33.22	19.63	40.77	15.1	13.66	15.1	4.08	1.21	4.9	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0	0

MONKEY	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	(Days) (Neg) Disp.
12920	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	109
12921	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Treated**
12922	0.01	0.01	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
12923	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	111
12973	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	104
12974	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	104
12977	0	0	0.01	0.01	0.01	0.1	0.01	0.01	0.01	0.01	0.01	0	0	0	0	0	0	92
12978	0	0	0.01	0.01	0.01	0.01	0	0	0	0	0	0	0	0	0	0	0	(43)Died

**= Treated with mefloquine 20 mg/kg

TABLE 34

DETAILED PARASITEMIA OF PASSIVE TRANSFER OF ANTI-EBA-175 REGION II PROTEIN MONOCLONAL ANTIBODIES TO AOTUS MONKEYS INFECTED WITH *Plasmodium falciparum* FVO

MONKEY	GROUP	Parasites x ccmm x 10^3														
DAY/PI		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
11969	1	0	0	0	0	0	0	0.01	0.01	13.5	19.6	15.1	27.1	30.2	57.3	90.6
12868	1	0	0	0	0	0	0	0	0.01	1.1	21.1	40.7	80	52.9	211.4*	
12867	1	0	0	0	0	0	0	0	0.01	19.3	96.6	247.6	549.6*			
12918	1	0	0	0	0	0	0.01	0.01	1	27.1	51.3	145.9	334.8	676.4*		
12930	2	0	0	0	0	0	0.01	0.01	1.6	57.3	138.9	131.3	281.8	300.9*		
12936	2	0	0	0	0	0	0.01	0.01	1.3	61.9	187	248	366	626.4*		
12917	2	0	0	0	0	0	0.01	0.01	0.01	6	6	24.1	66.4	92.1	229.5	283.1
12065	2	0	0	0	0	0	0	0.01	0.01	25.6	70.9	154	265.2	321.8*		

DAY/PI	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Days Neg. Disp.
11969	49.8	2	0.97	0.01	0.01	0	0	0	0	0	0	0	0	0	0	48(Died)
12868																
12867																
12918																
12930																
12936																
12917	187.2*															
12065																

*=Treatment with mefloquine 20 mg/kg

TABLE 35

DETAILED PARASITEMIA OF AOTUS IMMUNIZED WITH A PLASMID ENCODING REGION II OF EBA-175 FOLLOWED BY A EBA-175 RECOMBINANT PROTEIN BOOST AND INFECTED WITH *Plasmodium falciparum* FVO

		DAY PI	Parasites x ccmm x 10^3													
MONKEY	GROUP	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
12944	3	0	0.01	0.01	0.02	0.34	8	115.5	400.2*							
12941	3	0	0.01	0.01	0.02	0.68	40.7	112.5	121.1	198.4	122.3	93.6	63000	63040	51340	12080
12942	3	0	0.01	0.01	0.02	1.7	246	372	497.3*							
12834	1	0	0.01	0.01	0.01	0.01	2.2	2.8	9	147	60.7	259.7	200500	100500	85520*	
12945	1	0	0.01	0.01	0.01	0.01	10.5	2.4	18	36	77	61.5	82560	117000	98150	49550*
12946	1	0	0.01	0.01	0.02	0.01	46.8	58.5	132.8	401.9						
12948	1	0	OUT													
12947	1	0	0.01	0.01	0.02	0.01	54.3	60.9	386.5	816.0*						
12951	1	0	0.01	0.01	0.02	0.96	73.9	116.2	410.8							
12952	2	0	0.01	0.01	0.02	0.01	58.5	70.5	152.5	374.2	303.5	299	204110*			
12957	2	0	0.01	0.01	0.02	0.01	7.6	98.1	401*							
12959	2	0	0.01	0.01	0.02	0.19	63	22	416.1*							
12960	2	0	0.01	0.01	0.01	0.66	87	63.4	400.1*							
12966	2	0	0.01	0.01	0.01	0.01	27	60	205.5	501.9*						
12967	2	0	0.01	0.01	0.01	0.01	8	1.6	42	318.1	326.1	748.9*				
12992	CONTRO	0.01	0.01	0.01	0.01	0.01	31.5	55.8	96	400.0*						

MONKEY	DAY PI	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Days Neg.
12944	3															
12941	3	32110	7160	960	0.01	0.01	0.01	0	0	0	0	0	0	0	0	38
12942	3															
12834	1															
12945	1			DIED												
12946	1															
12948	1															
12947	1															
12951	1															
12952	2															
12957	2															
12959	2															
12960	2															
12966	2															
12967	2															
12992	CONTROL															

*=Treatment with mefloquine 20 mg/kg

TABLE 36

DETAILED ACTIVITY OF ARTELINIC ACID* (WR 255663; BP11387) VS ARTESUNIC ACID(BM 17174) AGAINST INFECTIONS OF Plasmodium falciparum FVO IN AOTUS MONKEYS

				Parasitemia x ccmm x 10^3											
		RX INITIATED		DAY PRE	DAY OF RX					DAYS POST RX					Days
MONKEY	DAY P.I.	DAY PAT.	mg/kg	RX	1	2	3	4	5	1	2	3	4	5	Neg.
13011	9	5	8*	2.4	59.8	24.1	0.46	0.01	0	0	0	0	0	0	11
13018	9	5	8*	4.2	63.4	1.7	0.34	0.01	0	0	0	0	0	0	8
13005	9	5	16*	2.7	64.4	21	0.46	0.01	0	0	0	0	0	0	19
13006	9	5	16*	4.4	121	61.5	1.7	0.01	0	0	0	0	0	0	20
13013	9	5	32*	2.5	45.5	18.1	0.38	0.01	0	0	0	0	0	0	23*
13016	9	5	32*	1.3	70	27.6	0.67	0.01	0	0	0	0	0	0	23*
CONTROLS															
13010	11	7	32*	146.4	325.5	118.5	54.3	18.01	0.01	0	0	0	0	0	24
13017	11	7	32*	164.5	578.2	244.5	78	61.5	19.1	6	0.01	0	0	0	26

*= Treated while still negative

TABLE 37

SUMMARY OF ACTIVITY OF ARTELINIC ACID* (WR 255663; BP11387) VS ARTESUNIC ACID(BM 17174) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO IN AOTUS MONKEYS

MONKEY	Daily dose x 5 days mg/kg	Response of parasitemia to Rx			Days from final Rx to parasite Clearance	Days from final Rx to recrudescence	Notes No. of days Negative
		None	Suppressed	Cleared			
13011	8*			X	1	12	11
13018	8*			X	1	9	8
13005	16*			X	1	20	19
13006	16*			X	1	21	20
13013	32*			X	1	*	23*
13016	32*			X	1	*	23*
CONTROLS							
13010	32*			X	1	25	24
13017	32*			X	3	27	26

*= Treated while still negative
Retreatment was carried out at next highest dose

TABLE 38

DETAILED ACTIVITY OF ARTELINIC ACID (WR 255663; BP11387) VS ARTESUNIC ACID** (BM 17174) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO IN AOTUS MONKEYS

				Parasitemia x ccmm x 10^3											
		RX INITIATED		DAY PRE	DAY OF RX					DAY POST RX					Days
MONKEY	DAY P.I.	DAY PAT.	mg/kg	RX	1	2	3	4	5	1	2	3	4	5	Neg.
13008	9	5	8**	3.04	144	62.4	1.6	0.01	0	0	0	0	0	0	8
12993	9	5	8**	4.1	103.5	49.5	2.1	0.01	0	0	0	0	0	0	19
13009	9	6	16**	3.2	85.6	9	0.38	0.01	0.01	0	0	0	0	0	21*
13014	9	5	16**	1.9	50	1.5	0.01	0.01	0	0	0	0	0	0	8
13012	9	5	32**	1.5	60.5	6	0.01	0.01	0	0	0	0	0	0	21*
13015	9	5	32**	3.3	65	1.9	0.01	0.01	0	0	0	0	0	0	21*

*= Treated while still negative

TABLE 39

SUMMARY OF ACTIVITY OF ARTELINIC ACID (WR 255663; BP11387) VS ARTESUNIC ACID** (BM 17174) AGAINST INFECTIONS OF *Plasmodium falciparum* FVO IN AOTUS MONKEYS

MONKEY	Daily dose x 5 days mg/kg	Response of parasitemia to Rx			Days from final Rx to parasite Clearance	Days from final Rx to recrudes-cence	Notes No. of days Negative
		None	Suppressed	Cleared			
13008	8**			X	1	9	8
12993	8**			X	1	20	19
13009	16**			X	1	*	21*
13014	16**			X	1	9	8
13012	32**			X	1	*	21*
13015	32**			X	1	*	21*

*= Treated while still negative
Retreatment was carried out at next highest dose

TABLE 40

DETAILED PARASITEMIA OF AOTUS MONKEYS IMMUNIZED WITH NATIVE AND SYNTHETIC EBA-175 AND MSP142 PLASMIDS FOLLOWED BY RECOMBINANT PROTEIN BOOST AND CHALLENGE WITH *Plasmodium falciparum* FVO STRAIN

MN		Parasitemia x ccmm x 10^3															
DAY/PI	GROUP	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
13034	1	0.01	0.01	0.01	3	34.5	22.8	613.0*									
13035	1	0.01	0.01	0.01	1.6	16.5	257.1	488.7*									
13037	1	0.01	0.01	0.01	1.8	28.99	260.9	930.1*									
13039	1	0.01	0.01	0.01	1	30	69	196	274	237	147.5	406.5*					
13056	1	0.01	0.01	0.01	4.1	64.5	204	440.9*									
13042	2	0.01	0.01	0.01	1.3	6.1	66	219.7	268.7	324	358	597.2*	227.4	DIED			
13043	2	0.01	0.01	0.01	0.02	1.5	19.2	38.2	101	16	61.5	110.2	195.1	400.0*	DIED		
13045	2	0.01	0.01	0.01	1.5	2	114.1	240.7	622.8*								
13064	2	0.01	0.01	0.01	960	770	57	95.8	564.7*								
13046	2	0	0	0	0.01	0.01	420	4.4	40.1	69.1	46.1	197	166	256.5	507.3*		
13047	2	0.01	0.01	0.01	2	18	111	413.0*									
13048	3	0.01	0.01	0.01	0.02	0.89	13.5	55	134.2	76.5	203.2	123.8	95.1	36.2	32.7	15.1	2.1
13049	3	0.01	0.01	0.01	1.8	2.8	51.1	277.8	454.5*								
13050	3	0.01	0.01	0.01	0.02	1.2	12	10.7	173.2	181.5	97.7	174.0**					
13052	3	0.01	0.01	0.01	0.01	0.01	11.5	38.2	134.2	202	362.4	400.7*					
13053	3	0.01	0.01	0.01	0.01	0.24	41.2	93.7	252.7	267.7	429.9*						
13054	4	0.01	0.01	0.01	0.02	0.43	7.9	22.6	165.7	180.8	434.8*	169.1	97.9	DIED			
13058	4	0.01	0.01	0.01	0.02	0.39	21.1	96.7	762.5*								
13059	4	0.01	0.01	0.01	3.8	4.9	149.5	421.2*									
13060	4	0.01	0.01	0.01	0.02	1	46.5	84.7	676.4*								
13061	4	0.01	0.01	0.01	0.02	0.01	2.9	25.3	131.2	156	265.7	537.3*					
13062	5	0.01	0.01	0.01	0.01	0.66	5.1	14.5	69.7	39.1	84.6	401.1*					
13063	5	0.01	0.01	0.01	0.89	0.99	26	54	144.7	114	205.3	365.7	400.9*				
13066	5	0.01	0.01	0.01	0.01	0.01	1.8	1.4	37	47	48.3	237.4	225.3	338.2	416.7*		
13067	5	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1.1	2.9	3.1	1.9	1	3.4
13068	5	0.01	0.01	0.01	0.01	0.01	0.24	0.53	3.6	13.5	13.5	54.3	69.4	70.9	77.5	89.0**	
13032	5	0	0	0	0	0	0.01	0.01	0.01	0.01	0.01	0	0.01	0.01	0.25	4.5	3.1
13070	6	0.01	0.01	0.01	0.01	0.01	3.7	83.4	646.2*								
13071	6	0.01	0.01	0.01	1.3	1.2	66	173.2	424.3*								
13073	6	0.01	0.01	0.01	0.01	1.4	4.9	12.4	111.7	487.1*							
13074	6	0.01	0.01	0.01	0.89	1	23.1	60.5	551.2*								
13075	6	0.01	0.01	0.01	0.01	0.01	19.9	72.2	173.5	153	459.0*						
13040	CONTR	0.01	0.01	0.01	0.99	3	24.1	125.5	384.7	331.2	388.9	410.0*					

* = Treatment 20 mg/kg Mefloquine

**= Treated with Mefloquine 50% Reduction in Hto.

TABLE 40 cont...

DETAILED PARASITEMIA OF AOTUS MONKEYS IMMUNIZED WITH NATIVE AND SYNTHETIC EBA-175 AND MSP142 PLASMIDS FOLLOWED BY RECOMBINANT PROTEIN BOOST AND CHALLENGE WITH *Plasmodium falciparum* FVO STRAIN

MN		Parasitemia x ccmm x 103																
DAY/PI	GROUP	22	23	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
13034	1																	
13035	1																	
13037	1																	
13039	1																	
13056	1																	
13042	2																	
13043	2																	
13045	2																	
13064	2																	
13046	2																	
13047	2																	
13048	3	8	0.01	0.01	0.01	0	0	0	0	0								
13049	3																	
13050	3																	
13052	3																	
13053	3																	
13054	4																	
13058	4																	
13059	4																	
13060	4																	
13061	4																	
13062	5																	
13063	5																	
13066	5																	
13067	5	21.1	17.1	17.1	24.1	16.6	14.4	13.7	36.2	53.2**								
13068	5																	
13032	5	9	10.5	10.5	13.5	10.5	11	2.5**										
13070	6																	
13071	6																	
13073	6																	
13074	6																	
13075	6																	
13040	CONTROL																	

* = Treatment 20 mg/kg Mefloquine

**= Treated with Mefloquine 50% Reduction in Hto.

TABLE 41. LIST OF PERSONNEL

Name and Position	% Effort
1. Nicanor Obaldía III, Principal Investigator,	100%
2. William Otero, Technician,	100%
3. Gloria Cisneros, Technician,	100%
4. Lionel Martinez, Technician,	100%
5. Maritza Brewer, Secretary	100%
6. Camilo Marin, Animal Care Taker	100%
7. Roberto Rojas, Animal Care Taker	100%
8. Temistocles Lao, Animal Care Taker	100%
9. Isaías Carrasco, Animal Care Taker	100%
10. Luis Carrasco, Animal Care Taker	100%
11. Víctor Herrera, Animal Care Taker	100%
12. Domitilo Rueda, Animal Care Taker	100%
13. Wenceslao Peña, Animal Care Taker	100%
14. Vicente Montenegro, Boiler Operator	100%